Principles of general physiology

Sir William Maddock Bayliss

PRINCIPLES OF GENERAL PHYSIOLOGY

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OF

GENERAL PHYSIOLOGY

BY

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Πάντα δοκιμάζετε
το καλὸν κατέχετε

Third Edition, Revised

WITH 261 *ILLUSTRATIONS*

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TO

E. H. S.

My Fellow-Worker.

PREFACE TO THIRD EDITION

No very great changes have been found necessary in this edition. The chief addition is a section on the capillary circulation, with regard to which important work has recently been published. Numerous references to further discoveries on questions already discussed have been inserted and the opportunity taken to revise with care the whole of the book. I wish to give my thanks to various correspondents who have kindly taken the trouble to inform me of errors.

The title of the book is still subjected to criticism. While admitting that a really "general" physiology should confine itself to laws of application to the whole of living nature, I find it impossible to find any name as suitable as that chosen for the manner of presentation adopted. Are laws that apply to animals but not to plants to be excluded from consideration on this account? And, if not, where is the line to be drawn between the "higher" and "lower" animals? As remarked elsewhere, it seems to be rather a question concerning the manner in which the subject-matter is arranged. This subject-matter is the same, however it is treated. It is obvious that general laws cannot be duly described and explained without concrete instances. I feel, however, that the plan adopted in my smaller "Introduction" is the more logical exposition of my point of view, although it would not be so suitable for the present work.

To return to the question of general principles, it cannot be too strongly insisted upon how important it is, if only in the saving of much mental effort at a later date, to obtain a thorough grasp of these main principles, and this applies to all sciences. It is often surprising how many of the details of apparently isolated facts fall into their places as natural consequences of a few general laws. In regard to medical education, for example, there is some need to guard against too narrow an interpretation of what is supposed to be of direct application to clinical practice. What is at the present time looked upon as pure abstract or academic science may turn out next week to be of vital importance. Take, for instance, the electrical phenomena of cell activity and their application in the electro-cardiogram.

There is another way in which the value of these principles needs to be kept in mind at the present day. There is, fortunately, a notable awakening to the value of scientific discovery. But it is apt to be chiefly appreciated in its practical aspect of the improvement of industrial processes or medical treatment. The necessary basis of this knowledge in previous experimental research into problems of no direct and obvious application is apt to be overlooked and, in consequence, suffers from lack of adequate

financial support. It will, I think, be obvious from the pages of this book that the greater number of the fundamental discoveries in physiology were made by men who were able to direct their own work in the way suggested by its actual progress. Such research must be perfectly free and devoid of external control. In making this statement I do not intend to undervalue what is done under the direction of another mind. Such work is very necessary in filling up the routine details in the complete building up of a great principle and also in the various modes of its application to practice. In this kind of research, what is called "team work" is, no doubt, of much value, and capable of wider extension than it has hitherto received; but it cannot replace the free unfettered excursions of the imagination of the individual worker, fruitless though these may often be. Such men of original and fertilising thought may not be common, but this is all the more reason that their activity should have the best conditions in which to produce its results.

At the same time it cannot be denied that there are some directions in which research is more profitable than in others, even from the point of view of general laws. Investigation may proceed in some of these directions too far for its results to fall into their places until other problems have been solved. Although they ultimately do so, it may happen that they are forgotten by the time when this might occur. A young inexperienced worker can often be usefully guided by the wider knowledge of a senior.

W. M. BAYLISS.

PREFACE TO SECOND EDITION

In the two years that have elapsed since the publication of the previous edition so many physiologists have been occupied with matters connected with the terrible war in which nearly the whole world is engaged, that no very great amount of work requiring reference in this book has been produced. The task of revision has not therefore been an onerous one, and the time needed could be found without neglect of other work.

I beg to thank my numerous friends and correspondents for pointing out errors overlooked in proof, together with actual mistakes, and for criticism in general. I hope that all these have been duly considered. Certain passages which were not as lucid as they should have been will be found to have been made clearer.

In the case of some aspects of the activity of muscle, of the kidney, and of the visceral nervous system, new facts or changes in point of view necessitated considerable rewriting. A new section on the transport of carbon dioxide in the blood has been added, and the reader will doubtless welcome the insertion of a portrait of Pasteur, unaccountably omitted from the previous edition.

The order of the chapters has, naturally, been the subject of some criticism. In any book dealing with so wide a domain a really logical order is impossible; whatever order is adopted, it is not to be avoided that knowledge of later chapters is occasionally presupposed. In a certain sense, each of the chapters of the present book may be looked upon as a separate essay, but there is, nevertheless, a definite sequence and connection between them. The only one which I am prepared to admit appears in a strange place is that on the Electrical Changes in Tissues. It would perhaps have been more appropriate if made to follow that on Electrolytes. The reader may give his attention to it at this place, if he wishes. The reason why it is placed where it is results from the fact that a knowledge of the electro-cardiogram is necessary in order to understand the heart.

A word seems requisite with regard to certain discussions contained in the earlier chapters, especially some of the questions of physical chemistry. The text-books on the subject do not always give sufficient treatment of those aspects which are of great importance in physiology. Original papers must be referred to in order to disinter some particular fact, and it seemed to me that I could save a certain amount of time and trouble for my fellow-workers. It is true that some of these matters may not seem to belong to a treatise on "General Physiology," but I know of no more appropriate name, and as far as I can find out these parts of the book have been found useful.

I would take this opportunity to correct an error in the former preface. Claude Bernard was Professor in the Collège de France, not in the University of Paris. The Collège de France, it will be remembered, was founded in 1530 by Francis I., and was at first somewhat opposed by the Sorbonne, but many of the most distinguished French scholars have occupied chairs in it.

In the previous preface, I referred to the view taken by Claude Bernard with respect to the position of vital phenomena in the world of experimental science. I would like to add a few further quotations here, because it seems that his position is sometimes misunderstood. They are to be found in the collection of lectures called "La science expérimentale." On p. 54 we read, "Pour le physiologiste et le médecin expérimentateur, l'organisme vivant n'est qu'une machine admirable, douée des propriétés les plus merveilleuses, mise en action à l'aide des mécanismes les plus complexes et les plus délicats." On p. 58: "Les propriétés de la matière vivante ne peuvent être manifestées et connues que par leurs rapports avec les propriétés de la matière brute, d'òu il résulte que les sciences physiologiques expérimentales ont pour base nécessaire les sciences physico-chimiques, auxquelles elles empruntent leurs procédés d'investigation et leurs moyens d'action." On p. 106: "Pour expliquer les phénomènes de la vie, le physiologiste expérimentateur s'adresse directement aux manifestations de ces phénomènes; il les analyse à l'aide des sciences physico-chimiques, qui sont plus simples que la physiologie, parce c'est toujours le plus simple qui doit éclairer le plus complexe." On p. 113: "Quant aux phénomènes de la vie, j'admets que ces phénomènes, considérés dans leurs formes diverses de manifestation et dans leur nature intime, ont à la fois une spécialité de formes qui les distingue comme phénomènes de la vie et une communauté de lois qui les confond avec tous les autre phénomènes du monde cosmique. Je reconnais en d'autres termes à tous les phénomènes vitaux des procédés spéciaux de manifestation; mais en même temps je les considère aussi comme dérivant tous des lois générales de la mécanique et de la physico-chimie ordinaires." On p. 118: "De ce qui précède, il résulte évidemment que le physiologiste, le chimiste, le physicien, n'ont en réalité à considérer que des phénomènes de même nature, qui doivent être analysés et étudiés par la même méthode et réduits aux mêmes lois générales. Seulement le physiologiste a affaire à des procédés particuliers qui sont inhérents à la matière organisée, et qui constituent par conséquent l'objet spécial de ses études. La physiologie générale se trouve ainsi ramenée à être la science expérimentale qui étudie les propriétés de la matière organisée et explique les procédés et les mécanismes des phénomènes vitaux, comme la physique et la chimie expliquent les procédés et les mécanismes des phénomènes minéraux." And on p. 212 (after referring to the separation by Leibnitz of the body and soul, which were supposed to act independently of one another): "si nous pouvons définir la vie à l'aide d'une conception métaphysique spéciale, il n'en reste pas moins vrai que les forces mécaniques, physiques et chimiques, sont seules les agents effectifs de l'organisme vivant, et que le physiologiste ne peut avoir à tenir compte que de leur action."

The only interpretation that I can put upon these passages is that the reason why we make an independent science of physiology is because the

laws of physics and chemistry exert their influence in a specially complex system. At present we are unable to analyse the workings of this machine to more than a limited extent. We know, for example, that glucose supplied to a living cell is burnt up and that the energy set free is used for particular purposes; but how this happens is as yet beyond our comprehension. Nevertheless, each step in analysis results in reducing some further stage to simpler laws. When we obtain an electrical current from a fish, we make use of a more complex manner of production than that from a galvanic battery, but we are not justified in saying that the vital production of an electrical current differs in any more fundamental way from that by a galvanic battery than this does from that by a thermopile or a dynamo-machine. From the philosophical point of view, of course, we know neither more nor less of the essential "nature" of living processes than of those of chemical action or electricity.

W. M. BAYLISS.

1917.

PREFACE

In the preparation of courses of lectures dealing with various physiological processes I have found considerable difficulty, and spent much time, in the extraction from books and original papers, many of them not biological, of material of fundamental importance in the proper treatment of the subject. The mechanism of reactions in heterogeneous systems may be mentioned. It seemed to me, therefore, that the results of this labour might be of use to others, whose work does not allow them sufficient time to read articles which do not appear to bear upon their particular domain of science. In arranging these facts, however, it became manifest that a somewhat wider treatment would be of more value, so that the book might be of service to all desiring a general, elementary, treatment of what may be called "abstract" physiology, as distinct from the "applied" physiology required by the agricultural, medical, or veterinary student for the purpose of his profession. In extenuation of my conduct in producing a work on physiology for the use, as I venture to hope, of all those who have any interest in science, I should like to quote a few words by Huxley to be found in his address, "On the Educational Value of the Natural History Sciences" (Huxley, 1902-1903, p. 59—see Bibliography). He gives an answer to the question, "What is the range and position of Physiological Science as a branch of knowledge, and what is its value as a means of mental discipline?" as follows: "Its *subject-matter* is a large moiety of the universe—its *position* is midway between the physico-chemical and the social sciences. Its *value* as a branch of discipline is partly that which it has in common with all sciences—the training and strengthening of common sense; partly that which is more peculiar to itself—the great exercise which it affords to the faculties of observation and comparison; and, I may add, the *exactness* of knowledge which it requires on the part of those among its votaries who desire to extend its boundaries." One would like to add also, the great experimental skill demanded, owing to the complexity of the phenomena studied.

The name of "general" physiology, which I have chosen as my title, corresponds very closely with what my honoured teacher, Burdon-Sanderson, used to speak of as "elementary" physiology, defining it as "the study of the endowments of living material," from which he expected the greatest advances of the future to proceed (Burdon-Sanderson, 1911, p. 217). This is practically the same view as that taken by the great Claude Bernard, who was professor of "physiologie générale" in the University of Paris from the foundation of the chair in 1854 until he died in 1878 (see Bernard, 1866, p. 8). In the lectures which he gave he insisted on the fact that

physiology, being the science of life, is to be regarded as an autonomous and independent study; in other words, that it is to be cultivated for its own sake, and not merely for its applications to the practice of medicine. If we look at the subjects with which he dealt, and which were in part published under the name of "Leçons sur les phénomènes de la vie communs aux animaux et aux végétaux," we obtain some idea of what Bernard understood by general physiology. We find fermentation, nutrition, combustion, protoplasm, irritability and contractility, respiration, and so forth, all treated from a wide and comprehensive point of view.

A notable passage from Sprat's "History of the Royal Society" (1722, p. 245) is of interest in this connection. The book is, it may be remembered, in great part an apology for the existence of a society for the purpose of making experiments. "It is stranger that we are not able to inculcate into the minds of many men the necessity of that *distinction* of my *Lord Bacon's*, that there ought to be experiments of *light*, as well as of *fruit*. It is their usual word, *What solid good will come from thence?* They are indeed to be commended for being so severe *exactors* of *goodness*. And it were to be wished that they would not only exercise this vigour about *experiments*, but on their own *lives* and *actions*, that they would still question with themselves, in all that they do: what *solid good* will come from thence? But they are to know that in so large and so various an *art* as this of *experiments*, there are many degrees of usefulness: some may serve for real and plain *benefit* without much *delight*: some for *teaching* without apparent *profit*, some for *light* now, and for *use* hereafter; some only for *ornament* and *curiosity*. If they will persist in contemning all *experiments*, except those which bring with them immediate *gain* and a present *harvest*, they may as well cavil at the providence of God, that he has not made all the seasons of the year, to be times of *mowing*, *reaping*, and *vintage*." A particularly striking case of the practical value of pure abstract laboratory work is to be found in the electric waves of Hertz, which were referred to in the first edition of Karl Pearson's "Grammar of Science" as of no practical application, but before the second edition appeared, they were used for wireless telegraphy (see Pearson, 1911, p. 30). Again, Tyndall points out (1870, p. 43), in reference to the great practical use now made of Faraday's electrical discoveries, "that if Faraday had allowed his vision to be disturbed by considerations regarding the practical use of his discoveries, those discoveries would never have been made by him."

Although most of the problems treated in the present volume are common to all living organisms, a few are included on account of their importance to a very large number of organisms, notwithstanding the fact that they are not, strictly speaking, of a "general" nature. The fundamental properties of the nervous system may be instanced.

It will be seen that the scope of general physiology is not identical with that of comparative physiology. This latter is sometimes apt to become in great part a description of functions peculiar to certain lower organisms, even when they throw no light on the activities of the human body, which are, after all, the most vitally interesting and important problems presented to the physiologist. Practically all the questions dealt with by general physiology apply both to man and to all living creatures, animal, or plant. In

treatises on comparative physiology, copious details of alimentary or digestive mechanisms will be found, but no discussion of the general nature of the action of enzymes.

In speaking of higher and lower organisms, it is well to make it clear that no invidious distinction is intended to be made. Both are equally well adapted to their environments. The higher are so called because they are affected by a greater variety of changes in their environment and respond to these in a more complex manner.

A certain amount of repetition is unavoidable, since the same process has different aspects and, owing to the interaction and interdependence of the phenomena observed in the more highly developed organisms, it is impossible to avoid references in the general treatment to activities which are also described as parts of complex actions in later chapters. The reader who is unable to follow the meaning of the text in places in earlier chapters, owing to reference to matters discussed in detail in later chapters, will usually find in the index the pages on which this description occurs, and can make himself familiar with them before proceeding further. A better course would be to read the earlier chapters a second time, after the later pages have been mastered.

An elementary knowledge of physics, chemistry, and biology must be assumed, unless the book is to become altogether unwieldy. It is indeed impossible to insist too strongly on the importance of at least an elementary knowledge of these three basal sciences for every one, much more for those pursuing the study of any branch of science whatsoever. At the same time, it has been thought useful to enter into some detail with respect to conceptions with which the student of physiology frequently finds difficulty, such as catalysis, the tension of gases, and some of the laws of hydrodynamics.

Vital phenomena being essentially dynamic, the study of physiology consists in the investigation of changes. As Jennings (quoted by von Uexküll, 1909, p. 30) says, "It is of the very greatest importance for the understanding of the behaviour of organisms, to look upon them chiefly as something dynamic—as processes rather than as structures. An animal is something that happens." The velocity of reactions and the conditions affecting it, together with the energy changes involved, are, therefore, more essential than the chemical structure or physical properties of the reacting substances or the resulting products, although the knowledge of certain of these properties is, of course, necessary. To use an illustration, inadequate as it is, that of a petrol motor, the problem of the physiologist is analogous to that of the investigation of the amount of fuel consumed in relation to the work done, when the engine is working under various conditions. The greater number of the chemical and physical properties of the materials used in the construction of the engine are of no importance, such as the valency of the iron or the smell of the lubricating oil, while others are fundamental, such as the heat of combustion of the fuel and the insulation of the ignition circuit. Even the exact chemical nature of the fuel is of subsidiary importance, so long as it is sufficiently volatile, and capable of giving an explosive mixture with oxygen. Moreover, the precise form of many parts, such as the heads of bolts, is immaterial, just as many structural details of living organisms or the precise chemical composition of connective tissue have,

at all events at present, an insignificant physiological interest. In making this statement, it is far from my intention to undervalue in any way the work of the organic chemist or the morphologist. Structure is the indispensable basis of function, and all structures, chemical or morphological, will, no doubt, ultimately have their function assigned. But, in these pages, space cannot be spared for description of such as have no functional importance suggested up to the present.

The treatment of the subject in the way here attempted undoubtedly has its difficulties. Important points have most probably escaped reference. I shall be very grateful to readers who will inform me of these omissions, and also for criticism in general. I feel that I may, in some places, perhaps, have laid myself open to the charge of neglecting statements which are in opposition to the point of view adopted. I consider myself justified in certain instances in doing this, on account of the disagreement of these statements with a large mass of knowledge otherwise obtained, and in the belief that further investigation will explain the apparent contradiction. As Sir Thomas Browne says (1672, vol. i. p. 115): "For what is worse" (that is, than new knowledge being but reminiscence), "knowledge is made by oblivion, and to purchase a clear and warrantable body of Truth, we must forget and part with much we know. Our tender Enquiries taking up Learning at large, and together with true and assured notions, receiving many, wherein our reviewing judgments do find no satisfaction." In other cases of omission, my ignorance must serve as an excuse. But, as Bacon has well pointed out, truth is more likely to come out of error, if this is clear and definite, than out of confusion, and my experience teaches me that it is better to hold a well-understood and intelligible opinion, even if it should turn out to be wrong, than to be content with a muddle-headed mixture of conflicting views, sometimes miscalled impartiality, and often no better than no opinion at all. One is tempted to quote Browning:—

"Stake your counter as boldly every whit,
Venture as warily, use the same skill,
Do your best, whether winning or losing it,

If you choose to play!—is my principle
Let a man contend to the uttermost
For his life's set prize, be it what it will!

The counter our lovers staked was lost
As surely as if it were lawful coin:
And the sin I impute to each frustrate ghost

Is—the unlit lamp and the ungirt loin,
Though the end in sight was a vice, I say,
You of the virtue (we issue join)
How strive you? *De te, fabula.*"

("The Statue and the Bust"—*last lines.*)

But, at the same time, there must never be the least hesitation in giving up a position the moment it is shown to be untenable. It is not going too far to say that the greatness of a scientific investigator does not rest on the fact of

his having never made a mistake, but rather on his readiness to admit that he has done so, whenever the contrary evidence is cogent enough.

In the present book I venture to lay down no expression of opinion as to the problem of "Vitalism," although it is scarcely possible to hide my feelings on the matter. I take it that there is no serious difficulty as to the kind of phenomena to be classed as "vital," and no dispute as to what are the problems with which the physiologist has to deal. If asked to define "life," I should be inclined to do as Poinsot, the mathematician, did, as related by Claude Bernard (1879, p. 23), "If anyone asked me to define *time*, I should reply: 'Do you know what it is that you speak of?' If he said 'Yes,' I should say, 'Very well, let us talk about it.' If he said 'No,' I should answer, 'Very well, let us talk about something else.'" The great physiologist, in another place (1878, pp. 116-117), describes what seems to me to be the most profitable attitude to take with regard to the question of vitalism; he says, "There is in reality only one general physics, only one chemistry, and only one mechanics, in which all the phenomenal manifestations of nature are included, both those of living bodies as well as those of inanimate ones. In a word, all the phenomena which make their appearance in a living being obey the same laws as those outside of it. So that one may say that all the manifestations of life are composed of phenomena borrowed from the outer cosmic world, so far as their nature is concerned, possessing, however, a special morphology, in the sense that they are manifested under characteristic forms and by the aid of special physiological instruments." It must be remembered, of course, that the special systems referred to are not to be understood as outside the laws of physics and chemistry. All that we are justified in stating is that, up to the present, no physico-chemical system has been met with having the same properties as those known as vital; in other words, none have, as yet, been prepared of similar complexity and internal co-ordination. A further point, with regard to which Claude Bernard's attitude is far more inspiring than that of those who regard living things as in perpetual conflict with external nature, may also be given in a translation of his own words (1879, p. 67): "It is not by struggling against cosmic conditions that the organism develops and maintains its place; on the contrary, it is by an adaptation to, an agreement with, these conditions. So, the living being does not form an exception to the great natural harmony which makes things adapt themselves to one another; it breaks no concord; it is neither in contradiction to nor struggling against general cosmic forces; far from that, it forms a member of the universal concert of things, and the life of the animal, for example, is only a fragment of the total life of the universe." (See also Kropotkin's attractive book, "Mutual Aid.")

My object, then, is to discuss the physical and chemical processes which intervene in these phenomena, so far as they are known. It must be kept in mind that all the methods available for the study of vital processes are physical or chemical, so that, even if there were a form of energy peculiar to living things, we could take no account of it, except when converted into known forms of chemical or physical energy in equivalent amount. This fact was clearly insisted upon by Burdon-Sanderson (1911, p. 164). Where explanation on these lines fails as yet, I have usually been content to summarise the general laws of the

process, leaving it for the future to carry further the reduction to simpler laws. Nevertheless, I fear that I may in some cases have been unable to resist the temptation to suggest hypotheses, even where the experimental data are inadequate. May I venture to hope that some of these suggestions will help to indicate gaps and to excite research to fill them up? If so, any labour involved in the writing of this book will be amply repaid.

It should be unnecessary to point out that vital processes can only be investigated where they exist, that is, in the living organism, either as a whole or in its separate parts, when these can be prepared in such a way as not to interfere with their function, or, if so, only in a known manner. Such experiments, when vertebrate animals are concerned, are known sometimes as "vivisections," an objectionable and misleading name. I should not have thought it necessary to refer to this question, were it not that certain people, whom one might reasonably expect to possess better knowledge, appear to hold that the progress of physiological science is possible without such experiments. Vesalius stated that the simplest experiment on the living animal, as a rule, revealed more than a long study on the dead body. With another set of people, who see no value in physiology, and frequently also none in science of any kind, I have naturally no concern, except to remind them that a great artist like Leonardo da Vinci, whom they probably hold in some esteem, not only thought differently, but actually performed "vivisections."

Finally, nowhere is the admonition of St Paul to the Thessalonians (first epistle, chap. v., 21), which I have placed on my title-page, more necessary than in physiological work, "prove" (or rather "test") "all things, hold fast that which is good." Let me remind the reader, also, that the word translated "good" is κάλος, which also means "beautiful," and in the passage quoted implies "true." Let us try to imitate the ancient Greeks, and look upon all that is true as both beautiful and good. All science should be κάλή, and not, as to many narrow minds, essentially ugly, although possibly necessary. It is not always easy, however, to take this point of view. But some of the greatest artists of the past devoted much time to scientific investigations; Leonardo has been mentioned already, and Christopher Wren may be added.

With regard to the use of the word "good" as applied to experiments, the remarks of Claude Bernard (1875, p. 516) should be kept in mind by the physiological investigator: "In physiology, more than anywhere else, on account of the complexity of the subjects of experiment, it is easier to make bad experiments than to be certain what are good experiments, that is to say, comparable. This is the reason of the contradictions so frequent amongst experimenters, and it is one of the chief obstacles to the advancement of medicine and of experimental physiology."

W. M. BAYLISS.

UNIVERSITY COLLEGE, LONDON,
1914.

NOTE.—I may take the opportunity here to thank those authors and publishers who have kindly allowed the reproduction of certain illustrations. Those to which no name is attached are by myself and, for the most part, were prepared especially for this work.

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PRINCIPLES OF GENERAL PHYSIOLOGY

CHAPTER I

PROTOPLASM

ELEMENTARY PROPERTIES

AT the very outset of our studies we are faced by one of the most difficult problems with which the biologist has to deal, namely, the structure, chemical and physical, and the elementary properties of protoplasm. This substance is met with in all living cells, but in various degrees of differentiation into more specialised structures. In its simplest form, as seen in the pseudopodia of the

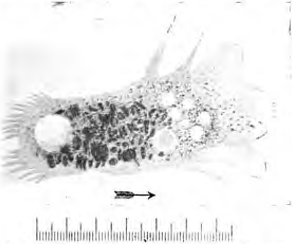

FIG. 1. AMŒBA PROTEUS (?).—Creeping in direction of arrow. Projecting in advance clear pseudopodia. The contractile vacuole is seen in the posterior end of the organism. Each division of the scale corresponds to 2·5 μ. (Leidy, 1879, Pl. iv, fig. 22.)

amœba or the leucocyte, it appears, even under the highest powers of the ordinary microscope, as a clear, colourless, jelly-like stuff, not showing any structure, but nevertheless keeping itself distinct from the fluid surrounding it, not mixing therewith, and also capable of changing its form in response to changes in its surroundings (see Fig. 1).

Fig. 2. Leucocyte of newt.—Fixed by a jet of steam directed on to the cover-glass. Stained with hæmatoxylin. Untouched photograph. Note the apparently homogeneous nature of the pseudopodial protoplasm. (Schäfer, "Essentials of Histology," fig. 67, Pl. 58.)

The structureless nature of protoplasm in its most elementary form is also, in certain cases, to be seen after fixation, as is shown in Fig. 2, in which it will be noticed that the external layer and the pseudopodia are completely clear.

Even in some of the simplest unicellular organisms special portions are differentiated off for the purpose of performing particular functions, the contractile vacuole, for example. In higher forms of life such parts are known as "organs," and exist as permanent structures; whereas the simpler creatures appear to possess the power of making organs as they are required. The food vacuoles, seen in Fig. 3, may be given as an instance. The water, taken in with food particles, forms temporary stomachs, as it were, into which digestive agents are secreted.

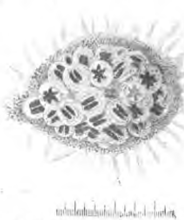

Fig. 3. Dinamœba mirabilis.—Interior filled with numerous cells of an alga, Didymoprium, enclosed in drops of liquid. The vacuoles are spherical, although the organisms included have irregular shapes. Each division of the scale corresponds to 2·8 μ. (Leidy, 1879, Pl. vii. fig. 3.)

"SUPER-MECHANICAL PROPERTIES"

This property of forming organs for temporary use, as required, is regarded by von Uexküll (1909, pp. 11-32) as demonstrating the impossibility of ever explaining protoplasmic activities on physico-chemical lines. This hopeless attitude does not seem to me to be warranted. Many of these "organs" are formed by the action of laws already known. For example, the digestive vacuoles are produced by the water taken in with the food, and owe their shape to surface tension; if digestive enzymes are present in the body of the protoplasm, they will naturally find their way into the vacuole. The pseudopodial changes of form are in relation to changes of surface tension and consistency of the outer layer of the protoplasm, as will be shown later.

In this connection an interesting experiment is described by Rhumbler (1898, p. 249). If a fine bit of glass rod be pushed against a drop of chloroform under water, it cannot be made to enter the drop; on releasing the pressure, it is immediately rejected. If, on the contrary, the rod be first coated with shellac, it is at once sucked in. As soon as the shellac is dissolved by the chloroform, the rod is thrown out again. I find it best to coat the glass with a filtered solution of shellac in chloroform, and then to dry it, since ordinary shellac is only partially soluble in chloroform. One might say that the chloroform will have nothing to do with substances which it cannot digest, and when a mixed food particle is presented to it and accepted, it digests a part and rejects the non-assimilable remainder. See also Rhumbler (1910, 1914).

My object in quoting this experiment is to call attention to the way in which quite simple combinations of well-known forces lead to the performance of complicated and apparently purposeful results. With respect to the similar process of the taking in of bacteria by leucocytes (*phagocytosis*), it is pointed out by Ledingham (1912, p. 324) that leucocytes, when floating freely, are spherical, and put out no pseudopodia unless in contact with some solid surface. Vigorous shaking of the mixture of serum, leucocytes, and bacteria does not affect the ingestion of the latter by the protoplasm, although there can be no pseudopodial activity. When chance contact takes place, there is taking in of the bacteria in a certain proportion of the encounters. The degree of phagocytosis is, therefore, controlled by the number of encounters in unit time. There is no indication of any kind of "seeking" on the part of the phagocytes. The process seems to be one in which surface tension is the chief factor. It is also obvious that, if the bacteria have been caused to agglutinate into clumps, each encounter will ensure the ingestion of a larger number of organisms at a time; hence the "opsonic index" merely shows the presence of something that affects the surface tension of the bacteria. The paper by Tait (1918) discusses the various ways in which surface tension intervenes in the phenomena shown by protoplasmic systems.

The "super-mechanical properties" of von Uexküll are also supposed to intervene in the activities of more differentiated structures, such as the muscle cells of actinia and so forth (von Uexküll, 1909, pp. 72 and 73). Although I am unable to follow this investigator so far as to deny all possibility of future explanation, there is no doubt that simple protoplasm presents very difficult problems. It is, in fact, at present, impossible to understand how a liquid, the properties of which protoplasm presents, as we shall see in a later page, can form organs at all. At the same time, it must not be forgotten that the composition of a liquid system is not of necessity the same throughout; a drop of oil may be floating in dilute alcohol. The various vacuoles in amœba do not all contain the same substances in solution, as will be seen in a later chapter.

Animals and plants are units in *time* as well as in *space*; they are compared to a melody in music, whereas machines are merely units in space. It is supposed that the human mind is unable to conceive such existences (see v. Uexküll, 1909, p. 28). But surely units in time are not wanting in the inorganic world. An atom of radium has arisen from uranium, through an intermediate element, at a certain time in past ages; it changes again, at a definite rate, into helium and niton, while the latter subsequently disintegrates into other elements.

According to Rutherford (1913, p. 668), the life of uranium is about 1,000,000 years; that of ionium, 100,000 years; that of radium, 3,000 years; that of niton, 5·55 days; that of radium A, 4·32 minutes; that of other intermediate products to radium F (polonium), 196 days; and it is probably finally converted into lead.

Moreover, it is characteristic of matter in the colloidal state (see Chapter IV.) not to be in permanent equilibrium—it is what has been called a "non-conservative system." It will become plain in later parts of this book how large a part colloidal phenomena play in the life of the cell. Van Bemmelen (1910, pp. 230-233) showed in 1896 that, if a preparation of colloidal silica as a moist jelly be taken and exposed to air containing various percentages of water vapour, the amount of water contained in the colloid varies continuously with the tension of the aqueous vapour. But the point of importance in the present connection is that, in certain regions of the curve, the amount of water present in the colloid at a given tension of water vapour is not the same if the silicic acid has previously been exposed to a lower tension, as it is if it has been exposed to a higher one. For example, if it has previously been in a drier atmosphere, and is then placed in one with a tension of water vapour of

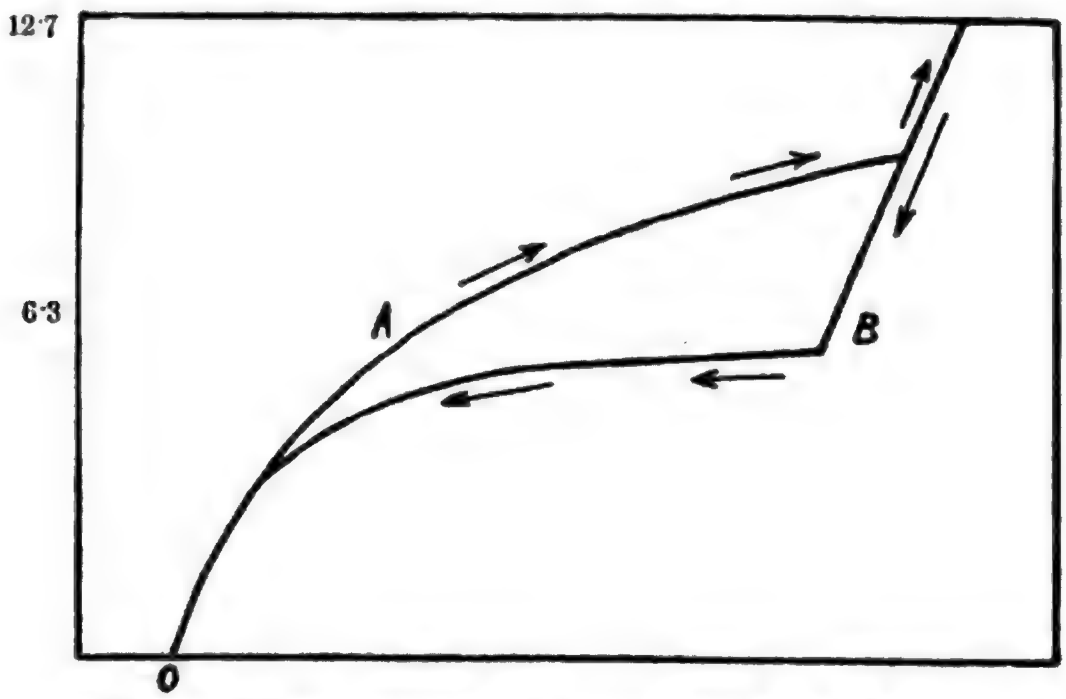

FIG. 4. WATER CONTENT OF A SILICIC ACID GEL, IN EQUILIBRIUM WITH DIFFERENT TENSIONS OF WATER VAPOUR.

Ordinates—tension of water vapour in millimetres of mercury.

Abscissæ—water content of gel: *A*, when exposed to increasing tensions; *B*, when exposed to decreasing tensions.

Showing "hysteresis." Inorganic systems have time factors and "life histories." Thus, from the water content corresponding to a tension of 6 mm. Hg (mid height of figure), we have information as to whether the previous history has been one of exposure to increasing or to decreasing tension of water vapour.

(From van Bemmelen's fig. 12, 1910, p. 247.)

6·3 mm. Hg, the water contained in the gel (A) (Fig. 4), after it has come into equilibrium with the gas phase, is less than one half of what it is if placed in the same atmosphere after previous exposure to one of a higher water vapour tension, say of 12·7 mm. Hg (B). Accordingly, if such a gel be placed in a water vapour tension of 6·3 mm. or thereabouts, information can be obtained of its previous history. The phenomenon here described is known as "hysteresis."

Again, it has been held that an organism differs from non-living matter in that its state at any moment depends not only on its previous history, but also on its future history. Here, also, similar conditions are not unknown in pure chemistry. The relative concentration of the components of a reversible reaction is determined at any time, not only by the initial state, but also by the final state, namely, that of equilibrium. The rate at which acetic acid and methyl alcohol combine to form the ester depends on the distance from the final state; if one may use a metaphorical expression, this final or equilibrium state is foreseen from the very beginning.

STRUCTURE OF PROTOPLASM

There is one fact about which there can be no doubt, that is, that protoplasm behaves as a liquid. This is shown by the spherical form taken by drops of watery fluid when they are enclosed in it (Figs. 1 and 3). These drops must therefore be free to take the form conditioned by surface tension, and hence no fixed or solid structures can be present to deform them, unless those structures are themselves freely movable. Further, when the fine particles, present in certain

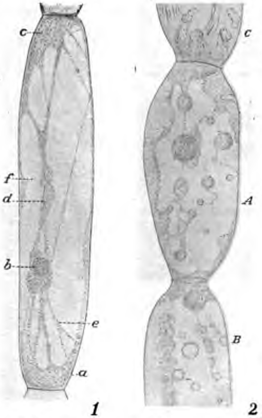

Fig. 5. Cells of staminal hairs of Tradescantia virginica.

1. Normal cell—*a*, cell wall; *b*, nucleus; *c*, protoplasm; *d*, wave of contraction in protoplasm; *e*, web-like plate arising from the fusion of two fine threads; *f*, moving bridge between two stronger protoplasmic currents. Length of cell, 0·3 mm.
2. Somewhat younger cells excited by induction shocks parallel to long axis. *A*, shocks of moderate strength; *B*, stronger shocks. In *C* the protoplasm is coagulated by rupture of cell and entrance of water. Length of cell *A*, 0·145 mm.

(Kühne, 1864, Pl. i. figs. 1 and 4.)

parts of protoplasmic organisms, are examined under the microscope, they are seen to be in constant movement. This phenomenon was first noticed by the botanist, Brown (1828, p. 359), and is therefore called "Brownian movement." Its nature will be discussed in Chapter IV., but its existence shows that the particles in question are suspended in liquid, and not held in a network or other kind of fixed structure. On the death of protoplasm, as Gaidukov points out (1910, p. 62), the movements cease, and a precipitation or coagulation, like the "setting" of gelatine when it cools, occurs; in the words of Graham, the hydrosol has become a hydrogel.

Further evidence in the same direction is afforded by the mode of response of protoplasm to an electric shock. When such a stimulus is sent through an amœba, it is made to draw itself together so that its surface shall be the least possible, in fact it becomes more or less spherical (Kühne, 1864, p. 32). This would be impossible if structures incapable of movement over one another were present. Similar changes are seen in the staminal hairs of Tradescantia (Fig. 5).

FIG. 6. CELL OF SPIROGYRA.

A, under ordinary illumination.

B, under brilliant dark ground illumination ("ultra-microscope").

The protoplasm appears clear and structureless in *A*; full of minute granules in *B*. Length of cell, 0·086 mm.

(After Gaidukov.)

Certain organisms known as mycetozoa in one stage of their life history, form masses of naked protoplasm. One of these, Badhamia, found on logs of decayed oak, was investigated by A. Lister (1888). It is usually full of the dark brown spores of the fungus on which it feeds, but it can be made to creep through wet cotton wool, which filters out the spores and clarifies the protoplasm. It is difficult to understand how a substance other than a liquid could be separated up into fine threads, which immediately run together again to form a mass like the original one, but devoid of the suspended bodies.

G. L. Kite (1913) states that Congo red and other dyes, injected into the interior of an amœba, diffuse rapidly throughout the protoplasm.

Although we are thus compelled to look upon protoplasm as a liquid, it shows under intense, oblique illumination ("ultra-microscope") that it is not homogeneous like water, or solutions of sodium chloride. On the contrary, it contains an immense number of minute particles, seen by this method (also called "dark ground illumination") as shining points, or diffraction discs (Fig. 6). There are present, therefore, substances in what we shall learn to recognise as the colloidal state. Similar conclusions are drawn by Mott (1912) from observations on living nerve cells by the same method.

Chambers (1917) distinguishes two kinds of particles visible in the ordinary microscope—very small ones, microsomes, and larger ones, macrosomes. The former are stable, the latter very sensitive to injury. "Mitochondria" are granules, apparently composed of albumin and lecithin, which stain with dyes containing diethyl-safranin, such as Janus green B (Hoechst). They are present in the living cell and undergo changes in its activity (M. R. and W. H. Lewis, 1914; Cowdry, 1916; Guillermond (plants), 1920). For the remarkable "Golgi Apparatus," consult Gatenby (1919).

It is unfortunate that the study of the phenomena presented by living cells is rendered difficult by the fact that so little can be seen by microscopic observation. A few words may, therefore, be useful here as to the nature of microscopic vision.

MICROSCOPIC VISION

Abbé, as is well known, attempted to reduce the formation of all images by the microscope to phenomena of diffraction. There is no doubt of the importance of this point of view, but, under correct methods of illumination, diffraction may be reduced so far that other modes of vision, refraction, and absorption, are preponderant. We will first consider *diffraction*. This phenomenon is due to the wave form in which light is propagated. It may be roughly described as the property of waves to bend round corners. Sound can be heard from a street at an angle to that in which it is produced, and the fact is sometimes disturbing, just as the corresponding phenomenon in vision through the microscope is. Another instructive fact is shown by the following case:—

Suppose a deep bay, narrowed at its opening to the sea by two stone jetties projecting from each side, and leaving only a small passage between them (Fig. 7).

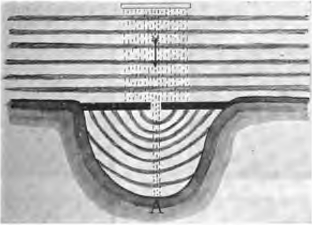

FIG. 7. DIAGRAM TO ILLUSTRATE DIFFRACTION OF WAVES OF THE SEA ENTERING A HARBOUR.

Waves approaching from the open sea pass through the gap, and spread out inside the harbour somewhat as represented in the diagram. An observer at A, supposing that he did not look at the opening, would obtain no evidence of its width from the waves arriving at his feet. On the other hand, supposing that a close fire of bullets were directed at right angles to the jetties, those that reached a cliff face at A would show the width of the opening.

In a similar manner light waves bend round the edges of objects, and diminish the sharpness with which images of these objects are formed on the retina. Blurred and incorrect definition of the boundaries of objects are only too frequently seen in published photographs of microscopic preparations. When such preparations have a regular pattern, such as the shells of diatoms, a number of totally distinct images may be formed according to the position of the objective.

Apáthy (1901, p. 514) describes the following experiment. A diatom of coarse structure, such as *Triceratium favus*, is observed by an apochromatic objective of 16 mm. focus, and ocular 8, 12, or 18. The substage iris is narrowed to 0·5 mm. in order to give a narrow cone of light. It will be found that no less than fifteen distinctly different images can be seen, as the objective is raised and lowered by the fine adjustment. These images are situated in the course of a movement of about 250 μ, whereas the total thickness of the diatom is only 4 μ, so that they cannot be due to differences of structure in the depth of the diatom itself. At the

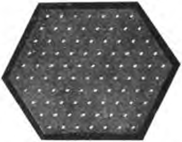

FIG. 8. REAL IMAGE OF ONE OF THE SECTIONS OF ABBÉ'S DIFFRACTION PLATE.—Photographed with Leitz $\frac{1}{12}$-in. oil immersion, projection oc. 4, Powell and Lealand condenser, full aperture of iris.

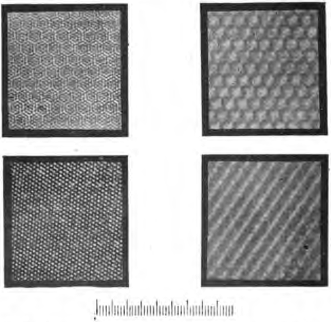

FIG. 9. FOUR DIFFRACTION IMAGES OF THE SAME PART OF THE PLATE AS FIG. 8.—Photographed with narrow illuminating cone. The images were taken first with Zeiss 16 mm. apochromatic lens, projection oc. 4. No condenser, plane mirror, iris aperture about 0·75 mm. The negatives were then enlarged to the same magnification as the real image in preceding figure, that is, 370 diam. Each division of the scale corresponds to 26 μ. The series of images were obtained by raising the objective through the space between real focus and one-third of a millimetre above. The lower right-hand image is that at the highest position (one-third of a millimetre) above the focus.

lowest and highest positions of the objective no real image can be formed, by refraction, within the tube of the microscope. As the iris is opened, the number of separate images diminishes. The same facts are even better shown by the use of Abbé's diffraction plate, as supplied by Zeiss. One of the figures on this plate consists of a series of rhombic clear areas, obtained by removing the silver coating by scratching a set of crossing lines and then preparing a photographic negative. The real structure is shown in the photograph of Fig. 8. With narrow iris, as in the previous case, a number of different images can be obtained, four of which I have photographed in Fig. 9. More details will be found in an article by J. W. Stephenson (1877, p. 87). The facts given here are sufficient to show that, by diffraction, structures can be seen which are quite unlike those actually present. It will be noted that the condition favouring their production is that of a narrow cone of illumination, due to a small aperture of the substage iris diaphragm. Some interesting photographs of diffraction images will be found in Edser's "Light" (p. 433). In these cases the images are more or less similar to the real objects.

The presence of different structures in a cell, even supposing that they are colourless, can be detected if they have *refractive indices* differing from that of the surrounding substance. Light rays will be deflected and give rise to darker and lighter spaces.

Colourless glass beads in air, observed under transmitted light by a low power lens, show dark and light rings; if immersed in oil of the same refractive index as themselves, they become invisible. Ordinary immersion oil is very nearly correct for this purpose.

Now most of the various structures in living cells possess very nearly the same refractive index, a fact which renders this mode of microscopic vision of limited use. Moreover, even when images are seen, they have only an indirect relation to the forms of the objects themselves, as is evident from the appearance of beads in air by transmitted light.

Suppose, however, that in the above experiment we take *coloured* beads. It will be found that, when immersed in oil, a beautifully clear and distinct image is obtained, whereas in air it is obscured by refraction. This shows what is to be aimed at in microscopic observation. Put shortly, we desire coloured objects, mounted in a medium of the same refractive index as themselves, and, to avoid diffraction, illuminated by a wide angled cone of light. This latter is obtained in "*critical illumination*," by which an image of the source of light is produced, in or very close to the plane of the object, by a substage condenser with iris opened as widely as the numerical aperture of the objective will permit. For details the textbooks must be consulted (for example, Spitta's "Microscopy," pp. 209-226). It is sufficient here to emphasise the fact that if, in a particular case, the light of "critical" illumination is too brilliant, it must not be reduced by narrowing the iris, nor by putting the condenser out of focus, but by the interposition of a screen of the necessary degree of opacity.

The mode of vision by absorption of certain components of light by coloured objects is therefore, *par excellence*, the method to be aimed at. Unfortunately, it is of but limited application to living cells, where so many of the constituents are colourless. There are, however, two cases where it can be used for such objects, and it is, of course, the aim of all histological staining processes. The two cases referred to are, firstly, photography by ultra-violet light, and secondly, intra-vital staining.

ULTRA-VIOLET PHOTOGRAPHY

Certain structures in the cell, although transparent to all visible wave lengths of light, and therefore colourless, are more or less opaque to ultra-violet light. So that if our eyes were sensitive to this light the objects in question would appear coloured. Now the photographic plate is sensitive to ultra-violet light, and Köhler (1904, pp. 129-165 and 273-304) has shown the possibility of photographing cells by this means. Fig. 10 gives photographs illustrating the fact. It will be noted that, although transparent and colourless to ordinary light, the nucleus is particularly opaque to light of the wave length of the ultra-violet. Unfortunately, the method has not as yet been made much use of, owing to the necessarily elaborate nature of the apparatus required.

Related to the method described above is that in which the *fluorescence* produced by ultra-

violet light when impinging on various substances is observed by the microscope. The colour of the light emitted by these fluorescent substances differs according to their composition, so that it may be possible to detect the presence in the living cell of substances otherwise invisible. Further information will be found in Chapter XIX., and in the papers by Stübel (1911), and by Heimstädt (1911).

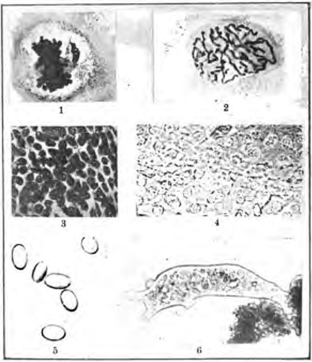

Fig. 10.

1 and 2. Dividing nuclei from gill plate of Salamander larva. Unstained, in glycerol. Photographed with ultra-violet light of 280 $\mu\mu$. The chromatic substance appears as if stained.

3. Edge of sternal cartilage of Newt. Living. Photographed with ultra-violet light. The nuclei are opaque.

4. The same. Photographed with ordinary light. The nuclei are transparent.

5. Red blood corpuscles of the Newt. Living. Photographed with ordinary light. Although oblique illumination was used, the nuclei are almost invisible. Traces of diffraction are seen around the corpuscles.

6. Amœba. Living. Photographed with ordinary light. The nucleus is just visible, but transparent.

(Nos. 1 to 3 after Köhler.)

INTRA-VITAL STAINING

The second method, that of staining the living cell, has been of much service. Ehrlich (1886) was the first to show that methylene blue stains living nervous structures.

A simple way of observing this fact is given by Michaelis (1902, p. 99). A short piece of the intestine of a mouse is placed for about half an hour to one hour in a solution of methylene

blue, containing one part of the dye in 20,000 of physiological saline. It is then slit open longitudinally and spread upon a slide with the serous coat uppermost. A glass cover is laid upon it with slight pressure. A little thick gum at the angles will serve to keep it in place. This preparation can be viewed even with an immersion lens. The plexus of nerves will be distinctly seen.

Subsequently, Ehrlich himself, followed by other workers, found that various

P Ehrlich

Fig. 11. Portrait of Paul Ehrlich.

other dyes are taken into living cells and deposited in them. Instances will be found in other parts of this book. Special structures have been found to be stained by particular dyes, and valuable information obtained (see the book by Goldmann, 1912). In the present state of knowledge of the physics and chemistry of the cell it is impossible to make definite statements as to the meaning of this specific staining of certain structures by particular dyes. Ehrlich holds that the dyes have special affinities for certain "side-chains" of the protoplasmic molecules; but recent work has shown that many other conditions also play a part, such as solubility, electric charge, diffusibility, and so forth.

It is difficult to see what purely chemical relationship can exist between complex, substituted diazo sulphonates, as a large number of these specific dyes are, and the chemical constituents of cells. Moreover, although methylene blue and other thiazines are specific vital stains for nerve tissue, certain safranin azo-dyes—diazingreen, for example—which have no chemical relationship to the former, are also vital nerve stains, while similar compounds of the same safranin series have no such property (Michaelis, 1902, p. 104). At the same time, one must not be too dogmatic where so little is definitely known. It will be necessary to discuss this question further in later chapters, and we shall see also that the conception of giant molecules in the chemical sense has very little evidence in its favour. For the present, it suffices to point out the fact that this specific affinity of dyes to particular structures exists, whatever may be its explanation. H. M. Evans (1915, p. 255) concludes that it depends on physical conditions, not on "chemo-receptors."

FIG. 12. DIAGRAM OF PART OF A CELL OF SPIROGYRA.

O, reticular coagulation of cell sap, as occurs after action of osmic acid. K, paths of the "dancing" particles (Brownian movement), as seen in the living cell; these paths are much longer than the meshes of the reticulum, so that the latter could not be present in life and must be a product of the action of the fixative.

(After Flemming.)

Much caution must be exercised in the interpretation of the results obtained by injection of dyes into living organisms. Very few are entirely devoid of poisonous properties, some are very toxic, so that when any one of these is found within a cell we have no means of knowing with certainty whether it found its way there while the cell was still alive, or whether it killed the cell first and then subsequently found its way to the inside, unless we have some criterion as to the vitality of the cell at the time when it is examined. This is to a certain degree possible in the case of unicellular motile organisms, but in the case of the tissues of the higher organisms the difficulty is obviously greater. Evidence may be obtained by the investigation of the permeability of the cell with respect to innocuous bodies, by methods to be referred to in Chapter V. It is clear that a dye cannot stain any constituent of a cell if unable to pass through the covering membrane, but it is not always possible to be certain that, when it does pass through, this happens without previously producing changes in the membrane itself. The dye may also be able to pass through the normal membrane, but may kill the cell when it reaches the internal structures. Statements are sometimes made with regard to the permeability of cells to dyes without taking due account of these possibilities. Many dyes, such as methylene blue, are reduced to colourless derivatives by certain cells while alive, but not when dead, so that reducing power in these cases may be used as a criterion of vitality (Michaelis, 1902, pp. 101 and 104). The living nucleus appears to be unstainable, so that when we see it begin to take up pigment we have warning of the death of the cell.

Neutral red is one of the least toxic of intra-vital stains.

Fixed cells behave to dyes quite differently from living cells; recently dead, but unfixed, cells have also properties in this respect unlike both those of fixed cells and those of living cells. It is probable that valuable information might be obtained from more detailed study of changes in dying protoplasm.

A portrait of Ehrlich will be found in Fig. 11.

FIXATION

Although protoplasm shows so little structure in the living state, it might be thought that, by use of fixing and staining reagents, more could be made

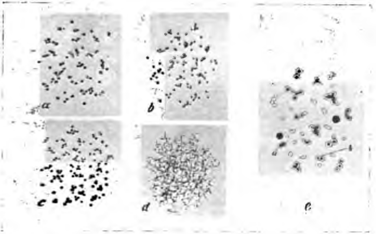

FIG. 13.

a, *b*, *c*, and *d*, forms of precipitate in 5 per cent. albumose, dissolved in 0·2 per cent. potassium hydroxide, when acted on by different reagents. Magnified about 600 diam.

a, with 1 per cent. platinum chloride.

b, with Flemming's solution.

c, with 0·5 per cent. chromic acid.

d, with Altmann's mixture of potassium bichromate and osmic acid.

e, 20 per cent. albumose, faintly acid, precipitated by mercuric chloride, stained with iron hæmatoxylin and then differentiated. Note fusion of globules to form aggregates (*a* and *b*).

(After Alfred Fischer.)

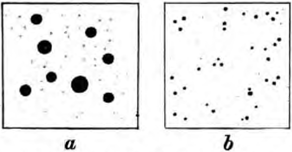

FIG. 14. DIFFERENTIAL STAINING OF GRANULES CONSISTING OF SAME SUBSTANCE, ACCORDING TO THEIR DIMENSIONS.

40 per cent. albumose, acted on first by 2·5 per cent. potassium bichromate, which caused turbidity, merely, in the solution. Subsequent acidification with acetic acid caused precipitation of granules of great variety in size. In *a* a preparation of this mixture was stained by Flemming's method in inverse order of dyes, namely, gentian violet, acid alcohol, safranin. *b* was stained in the usual order with safranin, acid alcohol, gentian violet. The darker granules in the figures must be supposed to be of a violet colour; the paler ones, red. In *a* the small granules are red; the larger ones violet. In *b* the opposite is the case. Staining with different dyes has, thus, no necessary relation to difference of chemical nature.

(After Alfred Fischer.)

out. Investigations, especially by Hardy (1899, pp. 201-210) and by Alfred Fischer (1899, pp. 1-72 and 202-336), have shown, on the contrary, that the structures obtained in this way are produced by the reagents used, and that quite different appearances are found in the same kind of cells according to the fixing substance used. A few facts will suffice to demonstrate this fact.

Flemming, in 1882 (pp. 50 and 51), noticed that the cell-sap of Spirogyra, which was a clear liquid containing particles in Brownian movement during life, became a rigid network after treatment with osmic acid (Fig. 12).

Alfred Fischer (1899, p. 34) takes a clear solution of albumose and acts upon it with various fixing reagents, obtaining various kinds of structures, as shown in Fig. 13.

Moreover, a homogeneous mixture of albumose and serum albumin, treated by Altmann's osmic and bichromate mixture, gave a structure consisting of granules embedded in a matrix of a fine reticular structure. These two structures could be stained in different colours by the usual histological methods (Fischer, *op. cit.*, p. 53, and Fig. 5 of the plate in his book).

Again, if a mixture of different sizes of granules of the same substance, say albumose precipitated by platinum chloride, be stained with methyl green and fuchsin, the large granules can be stained green, and the smaller ones red, or vice versa (Fig. 20 of Fischer's coloured plate. Similar figures are reproduced in monochrome in our Fig. 14).

Thus, after fixation, neither the form nor the staining properties give correct information as to the relationship of the constituents of the original system.

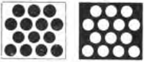

FIG. 15. DIAGRAM TO ILLUSTRATE RELATION OF PHASES IN COLLOIDAL SYSTEMS.

If the black be regarded as the solid phase, the white as the liquid phase, then *A* represents an ordinary hydrosol, such as that of gold, in which the solid particles are freely movable. *B* represents a gel of an alveolar or honeycomb structure, in which liquid drops are imprisoned by more or less solid walls, such as a strong solution of gelatine when cooled.

Hardy has shown (1899, pp. 163 and 184) that when substances similar to protoplasm in many of their properties, such as gelatine or egg-white, are acted on by fixing reagents, a separation of the solid from the liquid occurs, so that the former is obtained as some kind of a framework which holds the liquid portion (or "phase") in its interstices. Now there are two different kinds of structures which it is of some importance to distinguish from one another. When a 13 per cent. solution of gelatine is allowed to "set" by cooling, there is a separation of the solid from the liquid phase, but the latter cannot be squeezed out even by a pressure of twenty-six atmospheres. Whereas, if the jelly be fixed by formaldehyde, the liquid can be pressed out by hand. The two kinds of structures are known as vesicular and sponge-like. The essential difference is that in the former the liquid phase is in separate droplets each surrounded by a continuous film of the solid phase; in the latter, the two phases are reversed in position: the solid phase is in the form of a network of threads, while the liquid phase is continuous. A substance, therefore, which passes through a membrane of the former structure has to penetrate through the solid phase itself, while in the latter structure it can pass, although by a tortuous route, from one side to the other by means of the liquid phase only. In the language of colloid chemistry, one may also put it in this way: The dispersed phase in the one is in the position of the continuous phase in the other, and vice versa. The continuous phase is also called the external phase, and the dispersed phase the internal one. The relationship between the two forms of distribution may be made clearer by the diagram of Fig. 15, where the black represents the one phase and the white part the other phase. If black is solid and white is liquid, diagram B will represent the vesicular or foam structure, and A the network or reticular structure in section. The diagram A also represents an ordinary colloidal solution or an emulsion, if the black areas are supposed to be solid or immiscible liquid respectively. Clowes (1916) shows how these transpositions of phases can be brought about.

Different fixing reagents produce, then, different kinds of structure in gelatine. Alcohol or mercuric chloride gives a vesicular or foam structure and formaldehyde an open network, as we have seen.

In such systems as those under discussion the liquid phase corresponds to the "non-staining" substance of histologists.

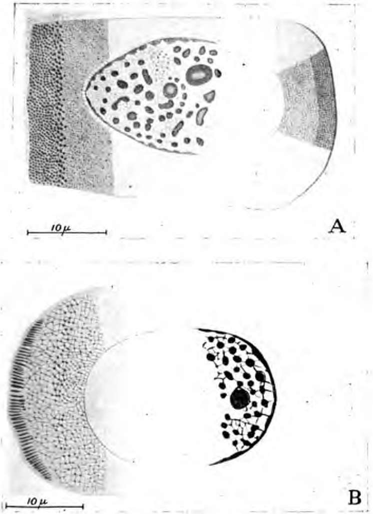

FIG. 16. CELLS OF GUT OF ONISCUS.—Stained with iron hæmatoxylin. Drawn with camera lucida.

A, after fixation with osmic vapour.
B, after fixation with mercuric chloride.

(Hardy, 1899, 1, figs. 18 and 19.)

If we turn to cells themselves, we find that the same cell shows a different structure of its protoplasm according to the fixing reagent used. For example,

Fig. 16 shows cells of the gut of *Oniscus*; A, after the action of osmic vapour; B, after mercuric chloride. Both structures cannot represent that of the living cell, and probably neither does.

Without the necessity of further details, it may be said that if we find vesicular (Bütschli) or network structures in fixed protoplasm, we are not entitled to assume the pre-existence of similar structures in the living state.

What, then, are we justified in concluding from the appearances presented by fixed tissues or cells? This much, of course, is clear, that there must have been something present in the living cell to give rise to the fixed structure; although, without further evidence, we cannot assume that there is any similarity between the two. Moreover, when we find in cells of the same kind, fixed, stained, and treated in the same way, the presence of something in certain cells absent from others, we may reasonably draw the conclusion that something has happened in the first which has not happened in the second. On the other hand, we must not assume that what we see is the same thing as the change which had taken place in the cell before fixation.

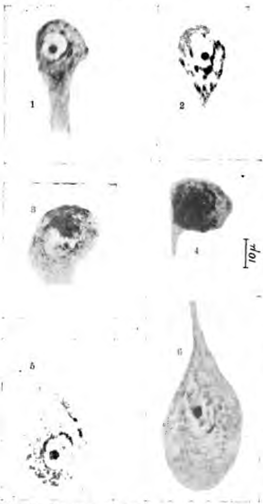

FIG. 17. CHANGES IN THE PURKINJE CELLS OF THE DOG'S CEREBELLUM RESULTING FROM MUSCULAR EXERCISE.

1. Normal cell.
2 to 4. Progressive increase in substance staining with methylene blue.
5. Later stage (fatigue), disappearance of the staining substance.
6. Still further stage of fatigue.

With the exception of No. 6, all were stained together, by Held's method, on a single slide.

(Dolley, 1909, figs. 1, 2, 3, 4, 9, 10.)

To take an instance, Fig. 17 represents the Purkinje cells of the dog's cerebellum, before and in different stages of fatigue, produced by muscular work. We notice that there is, at first, an increase of some substance which stains

deeply ("Nissl bodies"), but which afterwards disappears almost entirely. Whether this something, which appears and disappears, was originally present in the cells as aggregated masses, or uniformly diffused through the cell substance, we cannot tell. From the usual coagulating action of fixatives, especially from the separation of albumose and serum albumin in A. Fischer's experiments described above (page 14), the latter view is the more probable. Mott (1912) and Marinesco (1912, see page 470 below), in fact, have shown, by observations on living nerve cells under dark ground illumination, that there are neither Nissl bodies nor neurofibrils in the living state. Fine colloidal particles of a special nature are to be seen, but the protoplasm appears to have the uniform general nature of an "organised hydrosol."

We may also justifiably assume that, when we find structures in the same organ or cell, stained in different colours by a method of double staining, there is some difference between them, not necessarily of a chemical nature, and the structures had probably quite a different appearance in life.

DEHYDRATION AT LOW TEMPERATURES

A method has been introduced by Altmann (1894, pp. 27-29) which seems to offer possibilities for the investigation of the structure of cells without the use of fixing reagents. If a piece of tissue be allowed to dry at ordinary temperature, it is well known that it becomes so hard and horny that it is impossible to cut thin sections from it. And, even if this were possible, the structures would be altogether distorted. On the other hand, if dried over phosphorus pentoxide *in vacuo*, at a temperature so low that the salts of the tissue freeze out together with the water (forming a "eutectic" mixture—see the book by Nernst, 1911, p. 121), the cells are never exposed to the action of saturated salt solutions, which are formed when the tissue is dried at ordinary temperatures. A temperature of $-40°$ to $-30°$ C. is found to be low enough. The tension of water vapour at this temperature, although not absent, is very small, so that, even when accelerated by the use of a vacuum, the drying, even if very small pieces are taken, lasts for four days or so. Tissues so dried may be directly impregnated with toluene and paraffin at a temperature not exceeding 40° C. *in vacuo*. They cut as well as the best fixed and hardened preparations.

This fact I am able to confirm. My experiments were made by the use of the calcium chloride tank of a carbon dioxide freezing machine; the solution of calcium chloride was made of such a concentration that its freezing point was about $-35°$ C., so that by working the compressor all day the solution froze, and, being well insulated from heat, the temperature was maintained sufficiently low until the next morning. The object aimed at by Altmann was to compare the action of different fixatives on sections of the same piece of tissue. The sections were therefore exposed to these reagents at once, and no further difficulty was met with. My object, on the other hand, was to replace the water lost in the dehydration process, in order to examine the structure when unfixed, but a great difficulty was experienced owing to the immediate disintegration of the sections when brought into contact with water. It may be found necessary to allow water to be gradually taken up from ice at the same temperature as that at which the dehydration took place, allowing the temperature to rise very slowly. In any case, the method seems deserving of more attention than it has as yet received. Opportunities exist in laboratories provided with ice-making machinery.

That protoplasm is not killed by mere freezing, so long as it is brought rapidly down to the eutectic temperature, is shown by an observation by Kühne (1864, p. 101). Some Tradescantia hairs were rapidly frozen at $-14°$ in a platinum crucible, kept thus for five minutes, and then examined in water. After about ten minutes, the original condition of flowing protoplasm had returned and was still present twenty-four hours later. The facts have an industrial application in "brine-freezing," in which fish, etc., are rapidly frozen in brine at as low a temperature as possible. On thawing, the flesh regains its normal properties. Whereas if frozen slowly in air, ice crystals separate and are not reincorporated on thawing.

CHEMICAL NATURE

By chemical analysis, a great variety of organic compounds have been obtained from protoplasm. But it is obvious that it cannot be decided in this way whether they were combined together as a "giant molecule" in the chemical sense. The usual component elements of organic compounds are present, together with salts,

and as much as 85 per cent. to 90 per cent. of water. Proteins and "lipoids" are essential constituents. Carbohydrate is probably equally important.

A certain theory, that of "*biogen molecules*," has attracted many investigators (Verworn, 1903). According to this view, living matter consists of large molecules, with permanent central nucleus, and a great number of "side-chains," in the chemical sense. These side-chains are supposed capable of oxidation, reduction, methylation, and so forth. Under certain conditions, parts of the biogen molecules may be split off, but the essential phenomena of life are associated with changes in which these giant molecules take part as components of chemical reactions, taking place according to the ordinary laws of mass action, equivalent combining proportion, etc. In the thoughtful address of Prof. Hopkins to the British Association (1912, p. 220), which will be read with much profit, we find the following criticism, which seems to me to be entirely justified :—

"This view conceives of the unit of living matter as a definite, if very large and very labile molecule, and conceives of a mass of living matter as consisting of a congregation of such molecules in that definite sense in which a mass of, say, sugar is a congregation of molecules, all like to one another. In my opinion, such a view is as inhibitory to productive thought as it is lacking in basis. It matters little whether in this connection we speak of a 'molecule,' or, in order to avoid the fairly obvious misuse of a word, we use the term 'biogen,' or any similar expression with the same connotation. Especially, I believe, is such a view unfortunate when, as sometimes, it is made to carry the corollary that simple molecules, such as those provided by food-stuffs, only suffer change after they have become in a vague sense a part of such a giant molecule or biogen. Such assumptions became unnecessary as soon as we learnt that a stable substance may exhibit instability after it enters the living cell, not because it loses its chemical identity, and the chemical properties inherent in its own molecular structure, by being built into an unstable complex, but because in the cell it meets with agents (the intracellular enzymes) which catalyse certain reactions of which its molecule is normally capable."

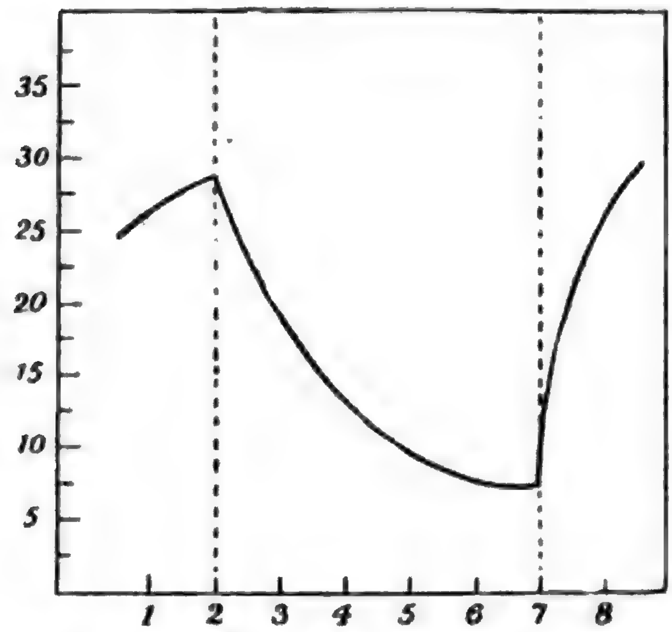

FIG. 18. CHANGE IN RESPIRATORY METABOLISM OF ASPERGILLUS NIGER, ACCORDING TO PRESENCE OF GLUCOSE.

Ordinates—milligrams of carbon dioxide per hour.

Abscissæ—time in hours.

At the second hour, the nutrient solution, containing glucose, was changed for tap water. The combustion processes immediately decreased to a low level, but were as suddenly restored, at the seventh hour, by addition of normal glucose nutrient solution.

There is apparently no store of food material. The steady small respiratory exchange in the absence of food is probably due to consumption of the organised structure of the cells.

(From experiments by Kosínski, 1902.)

If carbohydrate utilised by the cell becomes part of the protoplasmic molecule before oxidation, it is difficult to suppose that the nitrogenous part of the molecule would escape breakdown. That this is not so is shown by some experiments of Kosínski (1902), who grew Aspergillus in water, and on sugar solution. In the case of growth on pure water, the carbon dioxide given off falls at once, but to a value which is not zero, but about one quarter of that when grown on sugar. This production may possibly be that of the protoplasmic substance itself. When transferred to solutions containing sugar, the carbon dioxide rises *at once*, indicating direct utilisation without previous combination with "biogen" (see Fig. 18).

The use by bacteria of energy obtained from oxidation of inorganic sulphur, etc., is further evidence of non-oxidation of protoplasm itself. In many of these cases,

the presence of sugar appears to be actually injurious to the growth of the organism. Hydrogen gas can also be used as a source of energy. When hydrogen, oxygen, and carbon dioxide are present together, it is found that the oxygen is used up to oxidise hydrogen, and the energy so obtained enables the carbon dioxide to be used as a source of carbon: the three gases disappear simultaneously. Another interesting fact is that sulphur organisms also require a supply of carbon dioxide in the form of carbonate. See also Söhngen (1906) on methane as a food.

Evidence will be given in Chapter XX. and elsewhere against the supposed existence of *intra-molecular oxygen*. In fact, a view akin to that of Hofmeister (1901) is rapidly gaining ground. Hofmeister looks upon the cell rather as a laboratory, in which various operations are going on at the same time, being kept apart by membranes or partitions of some kind. Hopkins (1912, p. 220) advocates the existence of "interplasmic" reactions, in which substances formed by protoplasm are responsible for chemical changes in the cell. These reactions, then, take place in interspaces between the protoplasmic molecules, or rather molecular aggregates, themselves. Digestion in food vacuoles of *Amœba* may serve as an illustration of the process on a relatively large scale. Other reactions may occur in similar spaces, too small to be visible under the microscope. It seems that living matter is a complex of association processes of various types, in which physical forces play a large part, such as the surface condensation known as "adsorption" (Chapter III.), and also electrical charges. These forces control and regulate the course of the chemical reactions (Hopkins, 1912, p. 218). In any case it is evident that protoplasm, as it presents itself in such an organism as *Amœba*, is a system of many components or phases, solid and liquid, minutely subdivided and intimately mixed (see Gaidukov, 1910, pp. 61, 62, 74). In a certain sense, therefore, it may be said to have a structure, and the fact is of interest in connection with such chemical reactions as cease when the cell is ground up in a mortar. The cessation of the oxidation of lactic acid in muscle when chopped up (Fletcher and Hopkins, 1907, p. 284, and Harden and Maclean, 1911, p. 45) may be referred to. The effect produced by change of distribution of phases, as in Fig. 15, must also be borne in mind. Vernon (1912, pp. 210, 211) has been led, by his work on the effect of anæsthetics on oxidation in cells, to suggest the separation of cell constituents by membranes of a lipoid nature, a view similar to that of Hofmeister. Buchner (1903, p. 92) noticed that yeast cells containing glycogen showed no "auto-fermentation" as long as they were alive, but, when killed by acetone, this took place. Obviously, during life, the access of zymase and other enzymes to the glycogen is not permitted to take place.

A discussion of phase relations in protoplasm with respect to equilibrium and energy will be found in the essay by Zwaardemaker (1906, pp. 137-154).

As already pointed out above, protoplasm usually presents the characters of a liquid, but when dead it appears to take on a rigid structure, like melted jelly when it "sets," or egg-white when boiled. In this state it is no longer a liquid, and the Brownian movement of particles contained in it ceases, as they are held in a fixed structure.

An observation made by Gaidukov (1910, p. 58) suggests that such a change may take place temporarily during life; if so, this may be a means of localising chemical changes in particular parts of the cell. When protoplasm presents a free surface to watery fluids it is found to exhibit a continuous movement. In vegetable cells these movements are of a circulating or streaming nature. Now Gaidukov noticed, when observing the phenomenon in *Vallisneria*, that the streaming movement occasionally ceased and only a few of the particles showed Brownian movement. Presently, the Brownian movement began to reappear, and, as it increased, the streaming recommenced. I have been able to produce this reversible change from sol to gel by electrical stimulation (1920). It appears to be a common occurrence in states of cell activity, such as division and fertilisation (Chambers, 1917, 1, Leblond, 1919). Since Brownian movement is present in the flowing pseudopodia, the view that these are not due to cell activity, but to changes of surface tension outside the cell, is confirmed.

The "Biogen" theory is an effort to explain by purely chemical laws, facts which admit of simpler explanation if physical phenomena are also taken account of. It is

more than likely that chemical facts will sooner or later find their description in terms of molecular physics. The enormous molecules and aggregates of molecules which play so large a part in vital phenomena differ from simple small molecules in that they already begin to show the properties of matter in mass, especially those connected with the development of surface. This fact will be found to account for many otherwise puzzling phenomena, and cannot be ignored with impunity. Instances will be found in later pages of this book.

A few words are advisable with respect to the separation by chemical methods of various cell constituents. The view is held by Kanitz (1910, p. 234) that it is impossible to obtain any such substance in the form in which it existed in the living cell. He calls attention to the fact that, in the living cell, reactions must be supposed to be in continual progress, never actually arriving at equilibrium. A system in equilibrium is, in fact, dead, as will be seen better in the next chapter. But when a cell is acted upon by the reagents necessary to extract its constituents, the various reactions are supposed by Kanitz to be brought at once into equilibrium. The researches of Fletcher and Hopkins on lactic acid formation in muscle, already quoted, show that this is not necessarily the case. When muscle is heated to 40° C. so that it passes into heat rigor, the maximum amount of lactic acid to be obtained is formed (p. 266), about 0·52 per cent. as zinc lactate. When, on the contrary, the resting muscle is crushed under ice-cold alcohol, only 0·02 per cent. is obtained (p. 260). This is sufficient to show that the reaction producing lactic acid is stopped, practically at once, by destruction of the muscle structure at a low temperature. The manipulation requisite to extract it does not cause the reaction to proceed to completion. We may also call to mind that a reaction may be stopped at once by the addition of a chemical agent; as, for example, the hydrolysis of cane-sugar by the enzyme invertase, when a mercury salt is added, by which the enzyme is destroyed (see Chapter X.).

The following considerations, due to Hopkins (1912, p. 218), will show that the amount of a particular substance extracted from a cell is no index to its importance in the series of reactions going on in the living cell. The metabolism of the cell undoubtedly takes place in such a series of reactions that the products of one form the starting point of the next following. The various component reactions of this chain will almost certainly not progress at the same rate. Suppose, then, that the first component is kept constant in concentration by continuous supply, as will usually be the case. Then the amount of the products of each reaction present at any given moment will be in inverse ratio to the rate at which they change into the next member of the chain. It is clear that, in such a state of "*dynamic equilibrium*," the actual amount of chemical change taking place in each reaction must be the same; so that, if the rate at which any particular step is decomposed into the succeeding one is less than that at which it is produced from the preceding one, there will be a heaping up until the larger quantity reacting will compensate for the lesser rate of change. In symbolic form:—

$$K_1[A] = K_2[B] = K_3[C] = K_4[D] = K_5[E] = \text{etc.}$$

where K_1, K_2, K_3, K_4, etc., are the respective velocity constants of the reactions, and [A], [B], [C], [D], etc., are the corresponding concentrations, in accordance with the law of mass action. It is plain that, if K_1 is small and K_2 large, [A] must be large and [B] small, and so on. One important result of this fact is that, when a cell is killed, the amount of any particular compound present may be very small, although all the members of the chain of reactions may have passed through this stage.

REACTIONS OF PROTOPLASM TO EXTERNAL INFLUENCES

The movements of naked protoplasm have already been referred to incidentally. For more detailed description, memoirs such as those of Jensen (1902), Kühne (1864), or Ewart (1903), may be consulted.

The observation of the phenomenon, as shown in the staminal hairs of *Tradescantia*, should be made by every student. The hairs have only to be mounted in water under a cover-glass. The ordinary species, *T. virginica*, is grown in most gardens. If the flowers of the greenhouse species, *T. discolor*, are available, it will be easier to see the protoplasmic filaments, since the cell-sap is colourless, instead of being of a purple colour as in *T. virginica*.

The immediate cause of these movements seems to be changes of surface tension, produced either by outside influences or in the organism itself. The work of Rhumbler (1898, 1905) may be referred to.

A fact to be borne in mind, in discussing the behaviour of any organism to external stimuli, is that the response to similar stimuli is not always precisely the same. There is, so to speak, no fatal necessity about the reaction. This will be dealt with more fully in Chapter XVI., but the remark must be made here that we are not thereby compelled to assume the presence of a controlling "soul" or "Psyche." The *state of the organism* itself is by no means always identical. No stimulus, in other words, meets with a reacting system in precisely the same condition as a previous one did.

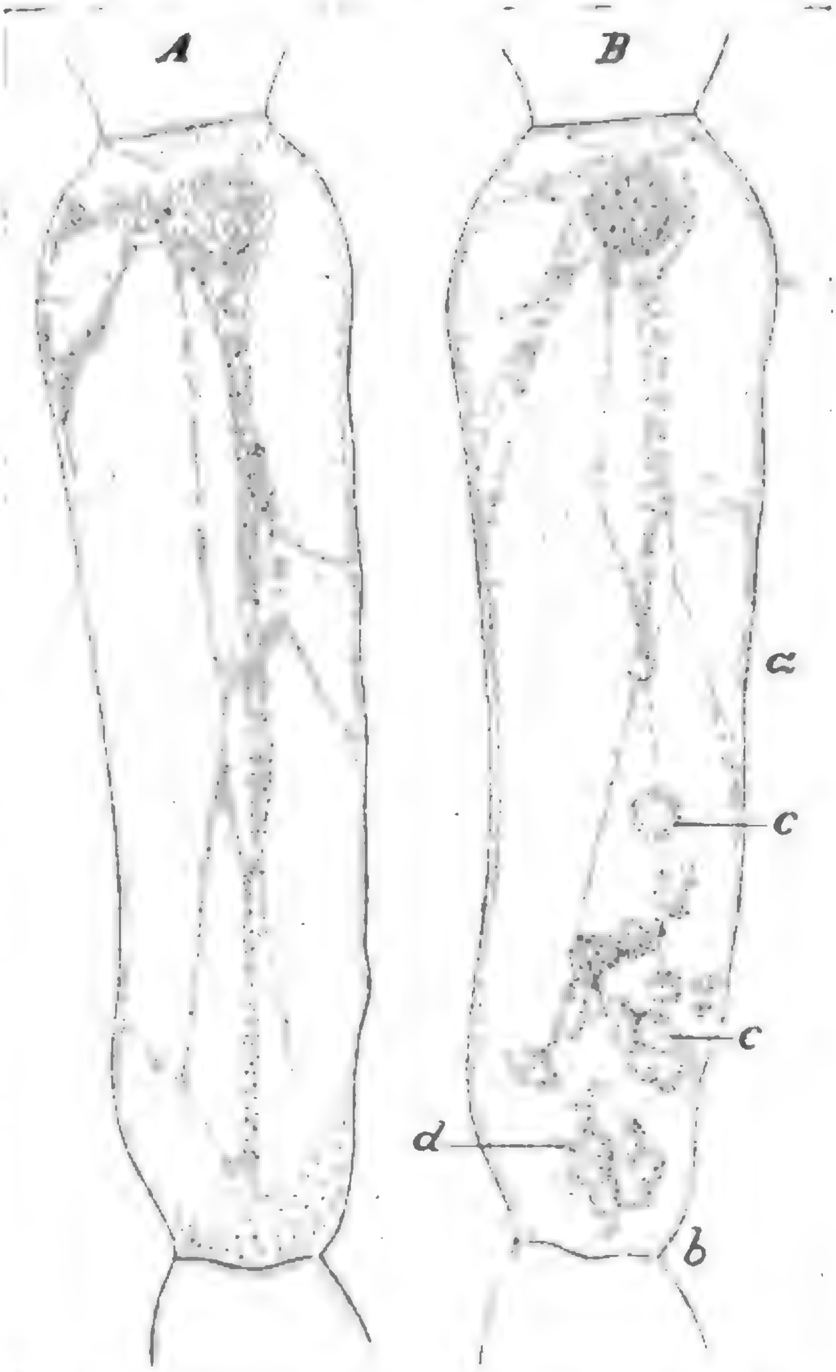

FIG. 19. CELLS OF STAMINAL HAIRS OF TRADESCANTIA, COPIED EXACTLY WITH CAMERA LUCIDA.

A, normal, in water.
B, the same cell, after moderate, local, electrical stimulation. The region of the excited protoplasm extends from *a* to *b*. *c*, protoplasm contracted to round lumps and balls. *d*, pale vesicles.
Length of cell, 0·2 mm.

(Kuhne, 1864, fig. 3.)

A sea anemone, which has been without food for some time, reacts rapidly to bits of crab meat, seizing them with its tentacles and pushing them into its gastric cavity. As repeated portions are presented, the reaction becomes gradually more inert, until finally no reaction is obtained at all. The presence of food in some way prevents the taking of more (Jennings, 1906, pp. 225-236). We are irresistibly reminded of a reversible, or balanced, chemical reaction becoming slower and slower as equilibrium is approached (see Chapters VIII. and X.). This is made the more striking by the fact that pieces of filter paper, which produce no chemical change in the organism, continue to be pushed into the gastric cavity as long as they are presented, although there is no room for them, and they are immediately disgorged.

Pages 111-127 of Jennings' Carnegie publication (1904), dealing with "Physiological States as Determining Factors in the Behaviour of Lower Organisms," should be read.

We have seen how portions of a protoplasmic organism, such as *Badhamia*, when separated by passing through cotton wool, subsequently coalesce again. The same thing occurs when the separate amœbæ, proceeding from germinating

spores, unite to form a plasmodium. On the other hand, it appears that the pseudopodia of an individual amœba, or other rhizopod, never unite with pseudopodia of another individual (Jensen, 1895, and v. Uexküll, 1909, pp. 16 and 38). The reason of this is not clearly understood. When the surface of such an organism comes into contact with food, it appears to soften and become sticky, so that the food substance adheres and is more readily taken in. The same thing seems to happen when a protoplasmic process comes into contact with another part of the same individual, but why it does not usually occur when portions of different individuals come into contact, is not easy to explain. Of course, no two individuals will be in precisely the same state, chemical and physical, at the same time, owing to different states of digestion of food and so on; but the power of discrimination possessed by protoplasm must be very great to appreciate these differences. There are, indeed, many other reasons for believing that living cells are extremely sensitive to minute changes in their environment.

When structures consisting of naked protoplasm, such as leucocytes or the streaming substance of vegetable cells, are exposed to an electric shock from an induction coil, their movements cease, and they draw themselves together into spheres or series of spheres as shown in Fig. 19 (see Kühne's description, 1864, p. 30). The way in which this effect is produced is not quite clear. The colloids are, as mentioned above, temporarily sent into the "gel" or coagulated state, but there seems to be also a kind of contraction of the surface layer in order to produce the spheroidal form of the protoplasmic masses. Some observations by Kühne himself (1864, pp. 31, 75, 95) point to the stoppage of Brownian movement, although the phenomena of colloidal systems were insufficiently known at the time to enable him to explain why this happened. Since he did not use dark ground illumination, the very small particles in the pseudopodia would be invisible and it is with these that the gelation can be seen apart from possible confusion with the cessation of the currents of protoplasm.

The effects due to the anode and cathode of the constant current can, in the main, be explained by electrolytic changes. Details of these effects are beyond the scope of this book, since they do not appear to throw much light on the problems with which we are concerned.

SURVIVAL OF CELLS

It has long been known that various organs of cold-blooded animals will continue their activities for a considerable time when separated from the rest of the body, but the corresponding fact in the case of warm-blooded animals has only been established by experiments of comparatively recent date. If artificial circulation of blood, sufficiently oxygenated and at the correct temperature, be maintained, it seems clear that the only experimental difficulty should be with regard to the lapse of time during which the organ is deprived of oxygen, during the necessary operative procedures.

An important step was taken when Locke (1901, p. 490) showed that the heart of the rabbit continued to beat for several hours if fed with a warm saline solution saturated with oxygen. The method has also been applied to the kidney and salivary glands, although it has, as yet, been found impossible to preserve all their activities. This will be discussed further when we are considering the mechanism of secretion. Other cases of isolated warm-blooded tissues, more especially smooth muscle, continuing their contractions immersed in similar solutions, will be found under the head of intestinal movements (Magnus). Blood vessels and the uterus can also be investigated by this method.

Further advance was made in 1907 by Ross Harrison (1907, p. 140, and 1910, p. 787), who found that cells separated from frog embryos and immersed in lymph continued to grow. Particularly valuable results were obtained

as regards the growth of nerve fibres from cells. Burrows (1911, p. 63) extended the method to the chick embryo, and Carrel and Burrows (1910) to

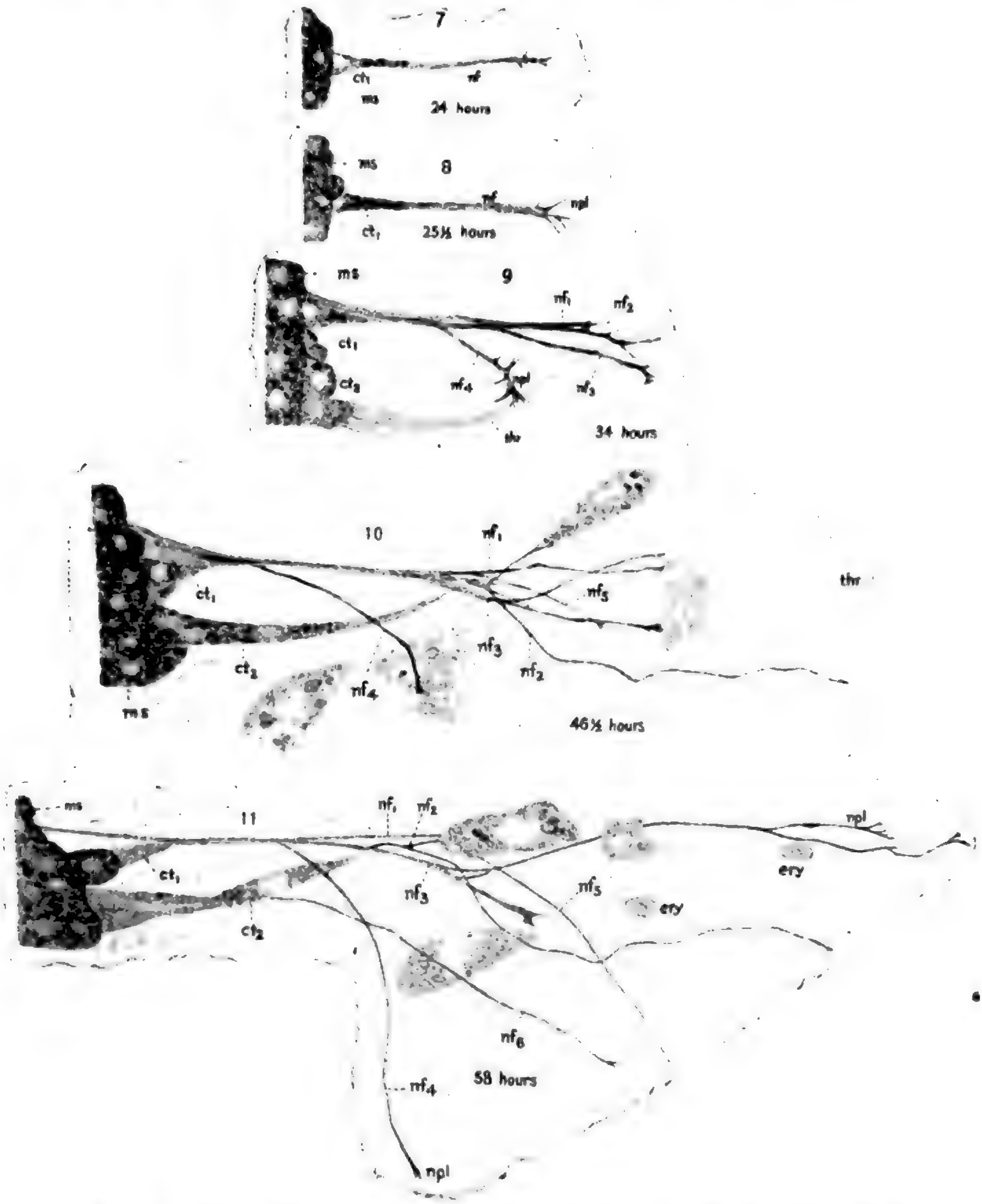

Fig. 20. Growth of nerve fibres in clotted lymph.—Medullary cord tissue of embryo *Rana palustris*, 3·3 mm. long. Lymph from *Rana pipiens*.

7. Apparently single fibre (*nf*) growing from a pointed cell (ct_1) which projects from a mass of cells (*ms*). One day after isolation of tissue, 28th April 1908, 12.25 P.M.
8. Same fibre, 2 P.M. Now seen to be double.
9. Same group of fibres, 10.25 P.M. Four distinct fibres (nf_1 —nf_4) now visible. Fibrin filaments (*thr*) were also present in the earlier stages, but omitted in sketches.
10. Same group, 29th April, 11 A.M. nf_5 possibly a branch of nf_1.
11. Same group, 10.30 P.M. Continuation of nf_1 and upper branch of nf_2 unfortunately left out of sketch. Note migration of cell ct_2. Identity of other isolated cells uncertain.

Total interval between first and last figures—thirty-four hours.

(Ross Harrison, 1910, Pl. 2, figs. 7-11; *Jl. Exper. Zoology*.)

the adult cat and dog, showing that fragments of various organs, such as kidney, spleen, bone-marrow, thyroid, cartilage, etc., placed in coagulated blood-plasma, form new cells of the appropriate kind. Renal tubules, for example, are to be seen increasing in length, and cartilage cells in number, while forming new cartilaginous substance. Fig. 20 represents the growth of a nerve fibre of the chick.

Fig. 21. Culture of kidney tissue in vitro.—From embryo rabbit at full term. After nine hours' culture. Fixed and stained with iron hæmatoxylin. The growing zone, at the upper and right-hand side, has a quite different aspect from the degenerated tissue at the lower and left side.

pl, plasma.
n, newly-formed tubules.
i, intermediate tubule.
g, glomerulus.
c, convoluted tubule.

The three latter are still to be recognised in the original tissue, although degenerated, while the tubules of the growing zone are all alike, and, at the outer boundary, undifferentiated embryonic cells alone are seen.

(Champy, 1913, fig. 6; *Rev. gén. des Sciences.*)

For the details of the method the reader is referred to the articles by Carrel and Burrows (1912), and by Carrel (1912). Further, Carrel (1913) finds that the culture medium is greatly improved by the addition of tissue juice of an adult or embryo animal, the younger the better, but this favourable effect is only shown when the tissue comes from the same species. Pieces

of the heart from a chick embryo continued to beat for two to three days after each removal to fresh medium, until unfortunately lost on the 104th day. Embryonic connective tissue could be made into subcultures repeatedly, since it grew so fast. After 14 months, tests grew to 30-40 times their bulk in 5 or 6 days. After 15 months the tissue was still living, although it had been transplanted 172 times.

It is important to remember that, when isolated tissues grow normally, we have satisfactory evidence of the preservation of their vital activity.

Some recent experiments by Champy (1913) have given interesting results. Confining our attention, to begin with, to the growth of kidney tissue, taken from an embryo rabbit at full term, we notice certain facts. In Fig. 21, fixed after nine hours of culture, we see a portion of new growth on the right and upper part of the figure, while the cells of the original portion are clearly degenerated; this degeneration appears to be due to failure of sufficient supply of oxygen. In the new growth there are tubules in the part first formed, but whereas, even in the degenerated state, it is easy to distinguish different kinds of tubules in the original tissue, the new tubules are all alike and of a primitive epithelial type (shown also in Fig. 22). As growth proceeds, we notice that the production even of these primitive tubules ceases, and there is merely a mass of indifferent cells, like those of the embryo before tissue differentiation commences.

If adult tissue is taken, such as smooth muscle, which no longer undergoes cell division in the organism, it is found that mitotic figures are produced *in vitro* and embryonic cells split off.

We see thus that differentiated cells, which undergo no further division as long as they are part of a complete organism, when cultivated in plasma outside of the organism, are set off on a course of multiplication, forming cells similar to those from which the differentiated cells were first formed. If it were possible to preserve these cells alive for a sufficient time, it would be extremely interesting to know whether they would ultimately be subject to differentiation into cells similar to those of the tissue from which they grew.

FIG. 22. INDIFFERENT RENAL TUBULE FROM THE GROWING ZONE OF A CULTURE SIMILAR TO THAT OF THE PREVIOUS FIGURE.—After seven hours. Fixed in Bouin's fluid, stained with iron hæmatoxylin.

c, cells arising from connective tissue.

(Champy, 1913, fig. 5.)

It seems that cells, when they have taken on special functions in the organism, are normally prevented, by some means, from continuing their primitive multiplication, and that, when this influence which restrains their growth is removed, they start afresh and produce simple embryonic tissue. There is significance in these facts in connection with the formation of malignant tumours.

The same investigator finds later (1914) that if the fragment of tissue happens to be composed of both epithelium and connective tissue, the new cells growing from the epithelium remain like those from which they grow; whereas, if by chance some of the epithelium leaves the connective tissue and grows towards the outside of the plasma, its cells lose their typical aspect and arrangement,

and cannot be identified as of epithelial nature. Similarly, in a fragment of retina, a typical proliferation of the connective tissue fibres does not occur as long as any nervous cells remain alive.

This mutual effect is not universal, it does not occur in the case of muscle and connective tissue. The facts, as a whole, tend to confirm the point of view expressed above as to the effect of one part of the organism on the growth of other parts.

Some further details, especially as to the absence of specific influence of the plasma of the same species of animal, will be found on page 288, and in Chapter XXIV.

The numerous and valuable results obtained by the investigation of chemical changes in surviving tissues and organs, such as muscle and liver, will be dealt with in later pages, when the particular functions in question are under consideration.

SUMMARY

Protoplasm in the living state has the properties of a liquid system, containing, however, particles of solids and droplets of immiscible liquids in a freely movable state. The protoplasm itself is structureless to the highest powers of the microscope, with ordinary forms of illumination. To the ultra-microscope it presents the characteristics of a colloidal system.

It forms "organs" for particular purposes; these organs appear and disappear, according to need.

But there is no necessity, at present, for the assumption of unknowable "supermechanical" properties in living cells. Many of the properties referred to can be explained by known laws, such as those of surface tension, while the time element itself is shown by inorganic colloids.

By fixing reagents, structure of various kinds, networks, alveoli, and so forth, can be produced. But these structures have no resemblance to the living condition. Obviously, they must be produced from constituents already present, so that certain conclusions are admissible from the examination of fixed cells.

There is very little ground for the view that protoplasm consists of "biogens" or "giant molecules," in the chemical sense. It is rather a complex of substances of various chemical natures and in various states of aggregation, associated together by forces of surface tension, electrical charge, and so forth. The liquid state enables an elaborate play of forces to take place. Chemical reactions can evidently proceed simultaneously in different parts of a cell, so that there is some mechanism by which one part is isolated from another part, at all events temporarily. After death, this separation ceases to be effective. The activities of the cell are regulated by reversible changes in the distribution of the phases of the complex heterogeneous system of colloids, crystalloids, and solvents.

For further information on the subjects dealt with in the preceding chapter, the following works may be consulted :—

LITERATURE

General Properties of Protoplasm.
Kühne (1864), pp. 28-108. Von Uexküll (1909), pp. 11-32. A. Lister (1888).

Structure of Protoplasm.
Hardy (1899). Gaidukov (1910). Rhumbler (1914). Chambers (1917).

Mitochondria.
Cowdry (1916).

Fixation of Cells.
Alfred Fischer (1899), pp. 1-72. Hardy (1899).

Movements of Protoplasm.
Ewart (1903). Hörmann (1898). Jensen (1902).
Rhumbler (1898 and 1905).

Survival and Growth of Tissues.
Ross Harrison (1907 and 1910). Carrel and Burrows (1910).

Methods of Investigation of Unicellular Organisms.
Schleip (1911), pp. 1-74.

The student is advised to read the preceding chapter a second time after having read the following eight or nine chapters.

CHAPTER II

ENERGETICS

THE most striking characteristic of living organisms is the perpetual state of change which they show, as will have been clear from the previous chapter. It is a matter of general experience that, in order to effect changes, work must be done. This capacity of doing work is due to the possession of something which is called energy, and is frequently defined in these very words.

LAWS OF ENERGETICS

There are two great laws dealing with changes of energy, known as the first and second laws of Thermodynamics or Energetics. The reason of the name thermodynamics, used in this connection, is that the laws were first arrived at, in the main, from considerations of heat energy. The first law tells us that, while energy may be of many kinds, kinetic, thermal, chemical, electrical, and so on, which can be converted into one another, there is never any gain or loss. This fact, derived from universal experience, is known as the "conservation of energy."

It may be noted here that the observation that energy of motion can be transformed into heat suggested the thought that the latter is itself a form of movement, and ultimately that the other forms of energy which can be derived from heat are also kinetic in nature, not excepting chemical energy itself.

The second law is somewhat more abstruse, and deals with the "quantitative relations which restrict the convertibility of energy," as Nernst puts it (1911, p. 16). Thus, "while external work and the kinetic energy of moving bodies can be transformed into one another completely and in many ways, and can also be converted into heat, as by applying brakes to a railway train in motion, the reverse change of heat into work is only possible under certain conditions." This is the principle of Carnot and Clausius in one of its forms.

For example, in the case of a steam engine, the part of the energy given out by the fuel which is available for work is given by the ratio of the difference of temperature between the boiler and condenser to the absolute temperature of the former; this means, of course, that only a certain part of the heat energy given out by the burning coal can be utilised even in the most perfect steam engine, unless the condenser is at absolute zero.

FREE ENERGY

The fact just referred to led to the important distinction made by Helmholtz (1882, p. 33) between "free" and "bound" energy. It is plain that, of the energy contained in a system, only that part which can do work is of value.

As an illustration, imagine a system of two similar copper balls, isolated completely from the surroundings, one of which is initially at a higher temperature than the other. The system as a whole contains a definite quantity of heat energy, given by temperatures and thermal capacities of the constituents of the total mass. If left to itself, a part of this energy will pass from the warmer to the cooler body, until both are at the same temperature. During this process a certain fraction of the energy transferred may be used to perform work. When the two balls have arrived at the same temperature, although no loss of energy has occurred, no more work can be got out of the system in itself, but only when brought into relation with another system at a lower temperature. In this state, so far as the system itself is concerned, its energy content is not free, but bound and useless.

A further important fact, also arising from experience, is that free energy always decreases, if it possibly can, but never increases. In the above illustra-

tion, energy passes from the hot body to the cooler one, so that the difference of temperature diminishes, and with it the free energy; the reverse passage from a cool to a hot body never occurs. This fact has various applications, as we shall find later. It follows from it, for instance, that if a process resulting in a diminution of free energy can take place, it will invariably do so. This principle was applied by Willard Gibbs (1878, pp. 216, etc.) to the investigation of the deposition of substances on the surfaces of bodies immersed in solutions of these substances, and will be discussed in the next chapter.

Clausius, at the end of a fundamental paper (*Pogg. Annalen*, cxxv. p. 400, 1865), formulates the two laws of energetics as follows:—

I. The energy content of the universe is a constant quantity.

II. The entropy of the universe is always striving to a maximum. The word "entropy" is here used as having essentially the same meaning as the "bound" energy of Helmholtz. The law is therefore equivalent to the statement that "free" energy is always striving to a minimum.

The fact, derived from universal experience, that free energy always tends to diminish, if it possibly can, is sometimes known as the "*principle of Carnot and Clausius.*" It was also enunciated, about the same time as the publication of the paper of Clausius referred to above, by Lord Kelvin (then Prof. William Thomson) under the name of the "Dissipation of Energy."

The principle has obviously a great practical, as well as philosophical, importance. It has been made by Ostwald (1912) the basis of a general rule of conduct, which he calls the "Imperative of Energetics." The rule may be translated thus: "Waste not free energy; treasure it and make the best use of it." The relation of the second law of energetics to the calculus of probabilities is discussed by Guye (1917). It may be noted that, according to Nernst, entropy is absent at the absolute zero of temperature, in the case of solids and liquids. Thus the disadvantageous position of heat, as compared with other forms of energy, is due to the distance from its zero value at which we work; while we can readily get a complete absence of electrical or chemical energy.

CAPACITY AND INTENSITY FACTORS

Another property of energy will be made clear by the following consideration. The work to be obtained from a stream of water depends not only on the height from which it falls, but also on the quantity of water flowing. A mere trickle, even from a considerable height, is of no practical use. Energy is composed, then, of two factors, which are known as the "intensity" and "capacity" factors, and is equal to the product of the two factors, properly chosen.

In the above case the distinction is obvious, height being intensity, and quantity of water capacity. In electrical energy, the intensity factor is difference of potential or electromotive force, while the capacity factor is current. In heat, the intensity factor is temperature, what the capacity is does not at once seem obvious. Sometimes the name "entropy" is used, as in the $\theta\phi$ diagram of the engineer, where one co-ordinate is the absolute temperature (θ), the other (ϕ) is the capacity factor, or "entropy," so that the area is the heat energy. It would be better, perhaps, to limit the word "entropy" to its original definition as given by Clausius, viz., the ratio of the "bound" energy to the absolute temperature.

It will be noticed that the intensity factors are what are called "strengths," whereas the capacity factors are of the nature of spaces or masses, so that the latter sum together when combined, while the former do not.

If a litre of water at 50° be added to a second litre of water at the same temperature, the energy content of the mixture will be twice that of a single litre, due to doubling the capacity factor; the intensity factor, temperature, on the other hand, is not altered.

The distinction between capacity and intensity factors was first made by Helm (1887).

These facts enable us to express the second law of energetics in a new way, viz.: in a closed isolated system transference or conversion of energy can only occur when differences of the intensity factor are present.

THEORY OF QUANTA

In ordinary cases of chemical combination, as is well known, additions are made by not less than one atom at a time; similarly, electric charges on ions

are added or removed by units of one electron at a time. The question naturally arises, are there similar phenomena in the case of energy? Now, in the consideration of the solid state of aggregation, certain phenomena have been met with which suggest that energy is dealt with in units at a time, in other words, that it cannot be divided into portions smaller than these units, called "quanta" by Planck (see p. 254 of Nernst's book, 1913). In the treatment of the solid state from the kinetic point of view, it is to be remembered that the molecules are only free to move or vibrate about a mean position, which does not change, contrary to what obtains in gases and liquids. Nernst (1913, p. 252) finds that the atomic heat of substances becomes very small as absolute zero of temperature is approached, and becomes practically *nil* at quite finite temperatures. In other words, the amount of energy imparted by the impact of vibrating molecules is not what the kinetic theory as applied to gases at ordinary temperatures would lead us to expect. The discrepancy is explained by the theory of quanta of Planck and Einstein, namely, that in the production of vibrations of an atom around its fixed position, as, for example, by the impacts of gas molecules, energy is taken up only in certain "quanta," and that these units are directly proportional to the period of vibration of the atom. For a freely movable gas atom this period is, of course, zero, so that in this case kinetic energy can increase steadily and the kinetic theory of gases remains unaffected. In the case of solids, a different state of things exists.

If this view be correct, it would follow that the curve giving the energy content, or partition of velocities between the atoms, instead of being a continuous one, would rise in a series of equal steps, each corresponding to a quantum of energy. A certain formula expressing atomic heats has been deduced by Einstein from this point of view, and, in the experiments made by Nernst and his co-workers, it has been found to be confirmed in the case of eight distinct elements. It applies also to the experiments in which the atomic heat of salts was determined by making use of the optical measurements of absorption bands made by Rubens. The absorption bands are taken as representative of the vibration periods of the atoms. See J. Rice (1915). Barkla (1916) finds that X-ray phenomena indicate that the quantum is a unit of atomic energy, rather than of radiation in general.

CHEMICAL ENERGY

Practically all energy available in the animal body is derived from the oxidation of food, and is, therefore, of chemical origin. It is very important to remember that chemical energy is readily transformed into other forms, without necessarily passing through the form of heat. In the various forms of primary batteries, the electric current, derived directly from the chemical reactions taking place, can be used to drive motors without any further change. The experimental facts concerning the relation of the heat produced in the contraction of muscle to the external mechanical work done show that the energy afforded by the chemical changes cannot pass through the stage of heat, since the proportion of work to heat is too high. The "efficiency" of muscle as a heat-engine would be 27 per cent. to 30 per cent. or more, according to various experiments. This would require, by the second law of thermodynamics, in a heat-engine, a difference of temperature between "boiler" and "condenser" of such a degree as to be incompatible with the life of cells. This fact was familiar to Fick (1882, p. 158), who makes the statement that the "chemical forces" must be used directly for mechanical work, and at the present time no physiologist holds the view that heat energy is a stage in the process.

What are the capacity and intensity factors in the case of chemical energy? Willard Gibbs (1878) suggested the name "chemical potential" for the latter, although "chemical affinity" is perhaps the better designation. This latter name, however, has been used somewhat vaguely. The capacity factor is clearly the quantity of a substance taking part in a reaction, that is the equivalent or combining weight, so that :—

Chemical energy = equivalent weight × chemical potential.

It may assist in understanding the meaning of chemical potential if we remember that, in a voltaic cell, chemical energy is directly converted quantitatively into electrical energy. Faraday showed that the quantity of electricity obtained is proportional to the amount of chemical change, so that the capacity factors of the two kinds of energy are proportional. Hence the intensity factors are also proportional, or electromotive force is a measure of chemical affinity. Faraday, therefore, was justified in regarding electrical force and chemical affinity as one and the same, as Mellor (1904, p. 26) points out.

Ostwald (1900, i. p. 249) regards chemical energy as being of as many kinds as there are elements ("Stoffe"). We have seen already how the intensity factor of energy in general never increases of itself; so that if the chemical potential of the products of a given reaction is higher than that of the reacting bodies, that is, when a substance is produced requiring to be supplied with energy, an endothermic reaction in fact, energy must be supplied from some extraneous source: it may be heat from neighbouring bodies or chemical energy from a concurrent reaction, involving fall of potential, in the same system. In the last case we have what is known as a "*coupled reaction.*"

While, therefore, there is only one kind of temperature, or two kinds of electromotive force, positive and negative, which can be increased or diminished by altering the magnitude of the forces producing them, chemical potential cannot be increased directly by the fall of potential in another reaction with dissimilar components.

Ostwald gives the following example:—Hydrogen peroxide is a body of higher potential than water or oxygen. Hence, in order to form it, the potential of oxygen must be raised, or the oxygen made "active." This cannot be done by any or every kind of reaction providing energy in the system, the neutralisation of acid, for example, but must come from a reaction such as the oxidation of phosphorus, in which part of the oxygen taking part in the reaction is made active by means of energy derived from the other part of the *same reaction* in which the potential of phosphorus is lowered by conversion to oxide.

The expression for the *maximal work* (A) of a chemical process is given by Nernst (1911, p. 658) as—

$$A = RT \log_e K,$$

where R is the gas constant, T absolute temperature, and K the equilibrium constant of a reversible reaction. All reactions can be treated as reversible. As it is put by J. J. Thomson (1888, p. 281), if we were able "to control the phenomenon in all its details, it would be reversible, so that, as was pointed out by Maxwell, the apparent irreversibility of any system is due to the limitation of our powers of manipulation." K, in the above formula, may be regarded as the ratio of two opposite reactions. It follows at once that the greater K is, that is, the nearer to completion the reaction proceeds in one direction, the greater the amount of energy available. In some cases we know the value of K, so that the free energy of the reaction can be calculated at once.

Nernst (1911, pp. 709-716, and 1913, pp. 741-753) has also put forward a new method which he thinks may lead to the determination of the free energy of any chemical reaction. Limits of space forbid its description here, and readers interested may consult the original (see also the work of Pollitzer, 1912).

It is held by Wegscheider (1912, pp. 223-238) that the maximal work to be obtained consists of two parts, one which is only to be got by making it to overcome external pressure, and is zero at constant volume; the other can be obtained in other ways, as electromotive force, for example. He gives formulæ for the minimum total work, for the electromotive force of chemical reactions, the dissociation of a gas, and a reversible gas battery.

The monograph by Helm (1894) may be consulted with profit.

SURFACE ENERGY

We shall see in the next chapter how the surface of contact of a liquid with a solid, a gas, or another liquid, with which it does not mix, the interface between any heterogeneous phases, in general, has the properties of a stretched film. It can therefore do work when this tension is able to decrease. Now if we consider the energy available in a living cell, we see that, although chemical potential can exert its full effect in a small space, the capacity factor of chemical energy needs considerable active masses in order that much total energy shall be afforded. In surface energy, on the other hand, although the

intensity factor can change but little, the capacity factor (*i.e.*, the area of surface) can vary very greatly within quite small spaces. Changes in the state of aggregation of colloids, by which their surface can increase or diminish a million fold, is, then, a potent factor in cell mechanics (see the remarks by Freundlich, 1907, p. 102).

LIFE AND ENERGY

In the picturesque language of Clerk Maxwell (1876, p. 93): "The transactions of the material universe appear to be conducted, as it were, on a system of credit. Each transaction consists of the transfer of so much credit or energy from one body to another. This act of transfer or payment is called work."

Now, as Benjamin Moore (1906, p. 1) rightly points out, it is just in this transfer of energy that the various activities which we recognise as peculiarly vital show themselves. The statement of Jennings as to the importance of regarding organisms as "dynamic" has been quoted in the preface to this book. In fact, a system in static equilibrium is dead. This fact, however, does not imply that chemical investigation of such a system is useless. Valuable information as to the energy changes involved can be obtained by comparing the chemical constitution of cells before and after performance of work.

There are many phenomena known which illustrate the peculiar activity of bodies in the very act itself of changing their energy content. The state of activity which can be conferred upon oxygen, by the oxidation of phosphorus or benzaldehyde, for example, appears to be connected with its change from a bivalent to a quadrivalent element, by which it gains electric charge. The active properties, however, are only manifested during, or immediately after, this change. The participation of electric forces can be shown by the steam-jet method of Helmholtz and Richarz (1890, p. 192). When a jet of steam issues from a fine glass orifice, it does not condense, so as to be visible, for a centimetre or so from the orifice. If bodies causing the formation of gas ions, *i.e.*, electrically charged molecules of gas, are brought into the neighbourhood of the jet, condensation occurs almost at the orifice itself, and the cloud becomes larger and denser. If a stick of phosphorus be brought near the jet, the effect is very marked. It was shown by the observers named that none of the chemical products of the oxidation of phosphorus have this property. The electrical phenomena are only to be seen during the actual oxidation process itself.

The active agent diffuses rapidly compared with currents of air; for, in the dark, the luminous vapours can be blown aside, without affecting the condensation of the steam jet. It is interesting to note that one of the authors of this paper was a son of the great Hermann von Helmholtz. This son, who showed much talent, unfortunately died before his father.

A remarkable fact of interest in the present connection was noticed by Straub (1907, p. 135) in the action of muscarine on the heart of Aplysia. The drug, at first present in higher concentration in the fluid in which the tissue cells are immersed, passes in course of time into the cells, until equal concentration exists within and without. But, although the drug can be shown to be present inside the cells by their action on another heart, its effect on the heart in which it is contained is no longer manifest. It is only during the actual passage into the cell, while its potential, so to speak, is different on the two sides of the cell boundary membrane, that it shows its characteristic effects.

Mention must here be made of the opinion of some writers that there is a special form of energy to be found in living matter, which is called by them "vital" or "biotic" energy. This is supposed to be convertible into equivalent quantities of the ordinary forms of energy, chemical, electrical, thermal, and so on, and vice versa. It is clear that no decision on the question can be arrived at until we have some instrument by which "biotic" energy, or, at all events, its intensity factor, can be measured, as the electrometer measures electrical potential, or the manometer, pressure of gas or liquid. For the present the assumption is purely hypothetical, and, as it seems to me, devoid of any purpose. It is to be noted that the modern adherents of this doctrine do not postulate anything more than a quantitative relationship between "biotic" and other forms of energy; in other words, the principle of the conservation of energy is supposed to hold even here.

The tendency of science is to greater simplification of the forms of energy; radiant energy has practically become a branch of electrical science, the inertia of matter has been explained by the properties of moving electrons, and Faraday had already felt the identity of chemical and electrical energy. It seems, then, somewhat retrograde to assume a new form of energy, especially as there is no urgent necessity for it. The resources of the known forms of energy are not altogether exhausted.

Further discussion of the application of the doctrine of energy to living organisms will be found in the essay by Zwaardemaker (1906).

Warburg (1914, pp. 256-259) calls attention to the fact that many cells, such as those of the central nervous system, the fertilised egg-cell, and nucleated red blood corpuscles, use energy in considerable amount, as shown by their consumption of oxygen, although they do no external work. It is evident that energy is required for some cell processes. Warburg suggests that it may be necessary for the maintenance of the "structure" of the cell, in the sense of keeping apart substances, which would mix by diffusion, the preservation of the properties of semi-permeable membranes, and so on, all in microscopic dimensions, or less.

HEATS OF COMBUSTION

The complete oxidation of such substances as fats and carbohydrates sets free a large amount of available energy. If this energy is all converted into heat, for the purpose of measurement, it is possible to obtain a number expressing the total energy content of any oxidisable substance. Numbers obtained in this manner are known as "*heats of combustion.*" They play a useful part in comparing the energy changes in various reactions.

The usual methods of determining heats of combustion will be found in the textbooks of Physical Chemistry (see that by Findlay, 1906, pp. 245-263). The adiabatic calorimeter of Benedict and Higgins (1910) appears to be a convenient and accurate form of apparatus. The name "*adiabatic*" is used in general for any process in which no heat is allowed to escape or be taken in. A gas, for example, may be compressed under such conditions that the heat produced escapes as fast as it is formed, so that the temperature remains constant; the process is "*isothermal*." If the heat produced by compression is prevented from escaping, the process is "*adiabatic*" and great rise of temperature may result. In the Diesel engine, the heat of compression is great enough to ignite the heavy oil used for combustion, although the process is not absolutely adiabatic, owing to cooling by the walls of the cylinder.

Heats of combustion, however, do not necessarily give the actual energy values of food-stuffs, as available in the organism. If converted into heat at once, only a comparatively small part can be utilised, even with large rise of temperature. Hence the importance of using the chemical energy of food in the way that will give most free energy. As A. V. Hill remarks (1912, ii. p. 511), "if it is shown that carbohydrate has, calorie for calorie of total energy, a higher proportion of free energy than fat has, this would have an enormous influence on theories of nutrition." This is given, of course, merely as an illustration of the necessity of due consideration of the difference between free and bound energy. In fact, Báron and Polányi (1913, p. 10), assuming Nernst's theorem (1913, p. 744), find that the free energy of the oxidation of glucose at 37° is 13 per cent. greater than the total energy, calculated from the heat of combustion. Heat must be acquired from surrounding bodies and converted to free energy.

Boltzmann, in one of his "Populäre Schriften" (1905, p. 40), points out how the "struggle for existence" of living beings is not for the fundamental constituents of food, which are everywhere present in earth, air and water, nor even for energy, as such, which is contained, in the form of heat, in abundance in all bodies, but for the possession of the free energy obtained, chiefly by means of the green plant, from the transfer of radiant energy from the hot sun to the cold earth.

THE GAS LAW AND OSMOTIC WORK

Boyle's law tells us that the volume of a gas is inversely proportional to the pressure, if the temperature is constant; and the law of Gay-Lussac tells us it is proportional to the absolute temperature, if the pressure is constant. In symbols:—

$$V = R\frac{1}{P}T \text{ or } PV = RT,$$

where V is volume, P is pressure, T is absolute temperature, and R is a numerical

quantity, called the "gas constant," whose value depends on the units in which the other factors are expressed.

This same law was shown by van't Hoff (1885) to apply to dilute solutions, and the theory of solutions based on the fact has had great effect on the progress of science. A portrait of van't Hoff in the year 1889 will be found in Fig. 23, in the year 1899 in Fig. 24. These portraits are given by the kindness of Prof. Ernst Cohen, of Utrecht.

When gases approaching their liquefying point, or concentrated solutions, are dealt with, the formula becomes more complex, since factors must be introduced on account of the molecules coming close together, so that their influence on one another, as well as the actual space they occupy, have to be taken into account. This question will be discussed in Chapter VI. In the present place, we will merely direct our attention to the expression which gives us the work done in compressing a perfect gas, or, by van't Hoff's theory, that done in concentrating a dilute solution. For simplicity, the temperature is supposed to be kept constant. This general equation will be found to turn up repeatedly in calculations involving considerations of osmotic pressure, such as the electromotive force of batteries, or the work done by the kidney.

FIG. 23. PORTRAIT OF VAN'T HOFF IN 1889.

(Jorissen and Reicher, 1912, p. 35. Reproduced by the kindness of Prof. Ernst Cohen, Utrecht.)

Suppose, then, that we take a volume, v, of a gas at a pressure, p, and that we compress it so that its volume is diminished by a minute fraction of its original volume, that is by dv. The work done is pdv. Further, if we diminish the volume v_2, which is occupied by one gram-molecule, to v_1, the total work done (A) is the sum of all the minute portions, pdv, between the limits of these two volumes. In the notation of the infinitesimal calculus:—

$$\mathrm{A}=\int_{v_1}^{v_2} pdv.$$

(Note that $\int$ is a lengthened *s*, the first letter of sum, and is used to indicate the totality of a process.)

Now $pv=\mathrm{RT}$, hence

$$p=\frac{\mathrm{RT}}{v} \text{ and } \int_{v_1}^{v_2} pdv=\mathrm{RT}\int_{v_1}^{v_2}\frac{dv}{v}.$$

(Note here that R and T, being constants, are not subject to integration, which of course applies only to variables.)

The value of this last integral is

$$\mathrm{RT}\log_e\frac{v_2}{v_1}.$$

For the complete solution, the textbooks must be consulted, *e.g.*, that of Nernst and Schönflies (1904, pp. 111 and 143) or of Mellor (1909, p. 254). A few words may perhaps be useful in enabling the reader to appreciate the meaning of the formula. The appearance of

FIG. 24. PORTRAIT OF VAN'T HOFF IN 1899.

(Reproduced by the kindness of Prof. Ernst Cohen, of **Utrecht**.)

the logarithm is due to the fact that the differential coefficient of the logarithm of x to base e is $\frac{1}{x}$, *i.e.*,

$$\frac{d \log x}{dx} = \frac{1}{x} \text{ and } d \log x = \frac{dx}{x},$$

and therefore, conversely, the integral of $\frac{dx}{x}$ is $\log_e x$, and that of $\frac{dv}{v}$ is $\log_e v$, or, when integrated between the limits of v_2 and v_1, is

$$\log_e v_2 - \log_e v_1 \text{ or } \log_e \frac{v_2}{v_1}.$$

Details of the way in which, by a simple application of the binomial theorem, the differential coefficient of a logarithm is obtained may be found in the books mentioned (Nernst-Schönflies, pp. 82-85, or Mellor, p. 51). We may note that the quantity e, chosen as the base of natural logarithms, is one of the most important in mathematics. As the sum of the infinite series:—

$$1 + \frac{1}{1} + \frac{1}{1.2} + \frac{1}{1.2.3} + \frac{1}{1.2.3.4} + \frac{1}{1.2.3.4.5} + \text{etc.};$$

its value can be obtained to as many places of decimals as required.

The *differential coefficient* of $\log x$ is the ratio of the amount by which $\log x$ increases when x increases by an infinitesimal fraction of its value, say it becomes $x + h$, to the increase h itself. That is, we want the value of $\frac{\log (x+h) - \log x}{h}$ when h becomes so small as to approximate to zero. When the expression is expanded by the binomial theorem, we finally arrive at another expression in which $\frac{1}{x}$ appears multiplied by $\log e$, *i.e.*,

$$\frac{d \log x}{dx} = \frac{1}{x} \log e.$$

There are many reasons for taking e as the base of a system of logarithms in dealing with mathematical formulæ, and when this is done, $\log e$ to the base e becomes unity. Our equation is then simply:—

$$\frac{d \log_e x}{dx} = \frac{1}{x}.$$

This digression into the region of pure mathematics is merely for the purpose of explaining the appearance of a logarithm in the expression for the work done in compressing a gas.

Looked at from another point of view, the work needed in each successive step depends on the result of the preceding step, hence the whole expression takes on a form in which this fact is taken account of. That is, it must be an exponential or logarithmic one, not a simple linear one.

Attention may be called to the frequent occurrence of processes whose magnitude at any given moment depends on how much of the process has been already completed, or, when an equilibrium is being approached, on the nearness to the end the process is. In the case before us, the work needed to cause the same actual diminution in volume of a gas increases the more the gas has been already compressed. Perhaps the simplest case is that of the absorption of light by a coloured liquid. Suppose that we allow 100 units of light of a certain wave length to enter the liquid and that, after it has passed through one centimetre, it has lost 0·1 of its original intensity and has become 90 units, or $100 \times \cdot 9$; after the next centimetre, this 90 units will have lost 0·1 of 90 and become 81, or $100 \times 0{\cdot}9 \times 0{\cdot}9$, *i.e.*, $100 \times 0{\cdot}9^2$, and so on. Hence, after passing n centimetres, its value will be $100 \times 0{\cdot}9^n$. Note that three layers do not absorb three times as much as one layer, but less, so that the value of the light transmitted is not 70 but 72·9. This law (that of *Lambert*) is used in the spectrophotometer, which has played so large a part in the investigation of hæmoglobin.

In such kinds of processes, then, we have to deal, not with simple linear relationships, but with exponential or logarithmic ones.

Other aspects of the question may be found in Newton's "*Law of Cooling*," one of the earliest cases to which the infinitesimal calculus was applied. Here the rate of cooling depends on the difference of temperature between the hot body and its surroundings, so that it steadily diminishes as the temperature difference becomes less; in theory, equality of temperature is attained only after an infinite time, asymptotically, as it is called, after the straight lines to which such a curve as the hyperbola continually approaches without actually reaching; this is due to the

fact that each succeeding portion of the curve moves towards the asymptote a little less than the previous portion did. In such cases as loss of heat, or the rate of a chemical reaction, we may regard the driving force as becoming less and less.

The increase of money lent at compound interest follows a similar law; for this reason, the general law in which a function varies at a rate proportional to itself, an exponential function, was called by Kelvin, "*the compound interest law.*" On this point, pp. 56-64 of Mellor's book (1909) will repay perusal.

The name "*function*" has just been used without explanation and it may be useful here to refer to some terms often met with in descriptions of phenomena from the mathematical standpoint. The volume of a given mass of a particular gas is different, according to the pressure to which it is exposed; but it is always the same, other conditions being unchanged, when the same pressure is applied. The volume of a gas is said to be a "function" of the pressure. A function, then, is a quantity which changes according to some definite law when another quantity, of which it is said to be a function, changes. This is expressed in symbols:—

$v=f(p)$, in the case of Boyle's law; or, generally, $y=f(x)$,

which means that, to every value of x, there is a determinate value of y. x and y are called "*variables.*" Any quantity which remains unchanged during a particular mathematical operation is called a "*constant.*" When the value of one variable depends on that of the other, as in the example given, the first is called the "*dependent variable,*" the second, the "*independent variable.*" Which of the two is chosen as the independent variable is a matter of convenience. In cases involving time as one variable, it is usually taken as the independent variable, since its changes are the most uniform. When the values of y are simple arithmetical multiples or fractions of those of x, so that the graph is a straight line, y is said to be a "*linear function*" of x. When y varies as a power of x, it is said to be an "*exponential function,*" and so on.

Speaking generally, the object of scientific research is to find out how one thing depends on another, in fact, what "function" the one is of the other.

To return to our main theme, we find that the work done in compressing a gas isothermally from the volume v_2 to v_1 is:—

$$\mathrm{RT} \log_e \frac{v_2}{v_1}.$$

Further, since, by Boyle's law, pressures are inversely as volumes, we have:—

$$\frac{v_2}{v_1}=\frac{p_1}{p_2},$$

and writing c_1 and c_2 for osmotic or molar concentrations of any two solutions as being proportional to p_1 and p_2, we have a formula which gives the work done in concentrating a solution from the value c_1 to c_2, as in the case of the kidney when secreting urine of an osmotic pressure different from that of the blood.

If c_1 and c_2 represent the concentration of an ion in two solutions in contact with electrodes of the same substance, we have the electromotive force of the battery, due regard being taken as to the units in which R is expressed. We shall see later how this fact is made use of to determine the real acidity of a solution, and how it is related to the electrical changes taking place in active organs.

For further details as to this important law, the reader is referred to the work of Nernst (1911, pp. 51 and 52), and the essay of Benjamin Moore (1906, pp. 21, etc.).

The practical bearing of the logarithmic form of the equation may be seen in the case of a concentration battery in hydrogen ions, as used for determining the true acidity or alkalinity of a complex fluid like blood, for example. If the relative concentration of the hydrogen ions in the two solutions compared is, in one case, as 2 to 1, and, in another case, as 10 to 1, the electromotive force in the second case will not be five times that in the first, but in the ratio of log 10 to log 2, that is, as 1 to 0·301, or about 3·3 times. Thus the actual E.M.F. of a battery, composed of a standard calomel electrode combined with a hydrogen electrode in one-tenth normal hydrochloric acid, is 0·394 volt, while if one-hundredth normal acid is taken, the value is 0·452 volt. It will be noted that the logarithmic form of the equation lessens the delicacy of the method.

MATHEMATICS IN PHYSIOLOGY

This is the most appropriate place to refer to the view taken by some, that the introduction of mathematics into biological questions is mischievous.

Huxley's (1902, p. 333) comparison of mathematics to a mill, which only gives out in another form what was put into it, is often quoted. At the same time we must not forget that this new form is much more useful than the original one.

Plato remarks, "If arithmetic, mensuration and weighing be taken away from any art, that which remains will not be much" ("Philebus," Jowett's translation, 1875, vol. iv. p. 104). Stephen Hales devoted himself to quantitative measurements in physiology and defined his point of view thus (1727, p. 2): "Since we are assured that the all-wise Creator has observed the most exact proportions, of *number, weight*, and *measure*, in the make of all things, the most likely way to get any insight into the nature of those parts of the creation, which come within our observation, must in all reason be to number, weigh and measure. And we have much encouragement to pursue this method of searching into the nature of things, from the great success that has attended any attempts of this kind." The Biblical passage referred to will be found in the beautiful 40th chapter of Isaiah, verse 12: "Who hath measured the waters in the hollow of his hand, and meted out heaven with the span, and comprehended the dust of the earth in a measure, and weighed the mountains in scales, and the hills in a balance?"

If it be admitted that our physiological methods are limited to those of physics and chemistry, further remarks are unnecessary. The value of mathematics in physics is plain to every one, and its value in chemistry becomes continually more obvious. As Arrhenius (1907, p. 7) points out, the expression of experimental results in a formula shows their relation to known laws in a way which is otherwise very difficult or impossible to attain. One is enabled to see whether all the factors have been taken into account and even an empirical formula may assist in deciding whether irregularities are due merely to experimental error or to some unsuspected real phenomenon in the process.

For example, the action of trypsin on a protein might be expected to follow the course of a uni-molecular reaction (see Chapter X.). Actually we find that the velocity constant calculated by the appropriate formula shows a continual diminution as the reaction proceeds. This fact leads us to look for the cause. In experiments on the influence of alkali we find that the activity of trypsin is, within limits, in proportion to the degree of alkalinity of the digest. We naturally look for diminution of alkalinity in the course of trypsin digestion and find that the production of amino-acids, especially the strongly acid di-carboxylic ones, is capable of producing a considerable change in the direction in question.

Possibly it may seem hard to add an extra burden to the already large equipment necessary for the physiological investigator. The reader will, no doubt, have been struck by the wide range of natural knowledge which has to be taken into account. At one moment we may be concerned with the movements of protoplasm in a vegetable cell, or the composition of the primeval ocean, and at the next, the work done in compressing a gas, the chemical properties of amino-acids, or the constitution of dyes.

In connection with the wide range of knowledge implied in the various problems with which physiology is concerned, it is interesting to remember that oxygen was discovered by a physiologist, Mayow, as we shall see in Chapter XXI., and many facts belonging to other sciences have also been brought to light in physiological investigations. On the other side, we may note that the function of the heart was practically discovered by an artist, Leonardo; the arterial pressure by a clergyman, Hales; the capillary circulation by a "bedell," Leeuwenhoek; intravenous injection by an architect, Wren; the nature of animal heat by a chemist, Lavoisier; the function of the green plant by a minister, Priestley; and so on.

A moderate amount of mathematics will probably have to suffice for most of us, enough to be able to understand and use the fundamental equations. But, since, as often insisted on already, vital phenomena are essentially changes, it will be obvious that the infinitesimal calculus, which deals with changing quantities, must be included, at least in its elements. It might indeed with advantage be allowed to take the place of much of the geometry and trigonometry

taught in our schools, as is well pointed out by Prof. Perry in his "Calculus for Engineers."

As a brief introduction, the first chapter of Mellor's "Chemical Statics and Dynamics" may be recommended. The admirable book of Nernst and Schönflies, of which unfortunately no English translation exists, may follow, and then, perhaps, Mellor's "Higher Mathematics for Students of Chemistry and Physics."

Fig. 25.—René Descartes.

(From the portrait by Franz Hals, in the Louvre Gallery.)

Experimental results can almost invariably be best expressed graphically, owing to the direct appeal to the eye.

The way in which algebraical formulæ can be represented by geometrical figures, or vice versa, was discovered by Descartes and published in his famous "Géometrie" in 1637. The co-ordinates when referred to two axes at an angle to one another, are accordingly known as "Cartesian co-ordinates." This system, with the axes at right angles, is that most commonly

used in representing experimental results in a graphic form. The fact should also be remembered that Descartes realised the import of his method as the commencement of "the expression by means of algebraical formulæ of continuously varying quantities" (Playfair).

FIG. 26. PORTRAIT OF JOHN NAPIER OF MERCHISTON.

(From portrait in possession of the University of Edinburgh.
From *The Merchistonian*, 1912-13.)

In other words, the history of the differential calculus may be said to begin with him. His portrait will be found in Fig. 25.

If the reader attempts to follow the reasoning given by Descartes himself, he will find it a difficult task. It seems as if the philosopher did not wish that his opponents, of whose mental capacity he had a very small opinion, should understand him too easily. Accessible editions of Descartes' works will be found given in the Bibliography at the end of the present work.

The experimenter, who uses a slide rule or a table of logarithms to diminish his arithmetical labours, should often feel grateful to the inventor of logarithms. This was **Napier** of Merchiston, whose portrait will be found in Fig. 26. Merchiston Tower is seen in **Fig. 27.** For most purposes, the short straight form of slide rule gives sufficient accuracy. If a greater number of significant places is required in the result, the spiral form of Fuller is very convenient in use. It is made by Stanley. The Handbook to the Exhibition at the Tercentenary of Napier, published by the Royal Society of Edinburgh in 1914, will be found useful in connection with the history and use of logarithms, as well as with other aids to calculation.

There is sometimes an unfounded prejudice against smoothed curves, but, if the data show any sort of regularity, the course of the phenomenon is more accurately shown by such a curve, since it eliminates accidental errors. It may be useful to describe the method, slightly modified from that of Ostwald-Luther (1910, pp. 28-30), which I have found the most convenient one for drawing curves for reproduction. The experimental values are first marked by + at the intersection of the co-ordinates, given appropriate values, on squared paper; a curve is drawn as smoothly as possible by hand, using a pencil, through these points. The paper is pasted on to a piece of moderately thick cardboard, which is then cut with scissors along the curve, so as to obtain a template. The movement of the hand in this operation is very regular, being sensitive to the least deviation from a regular course. Ostwald states that the co-ordination of hand and eye is sensitive to the second, or even third, differential coefficient. This template is used to draw a curve in pencil on Bristol board, which curve is then inked in by means of a French curve or a flexible curve. (The best is the "J. R. B." made by Harling, Finsbury Pavement.) It will be plain that the larger the scale, within limits, to which the curve is drawn, the better it will look when reduced for publication; the slight inaccuracies in the use of the French curve will be invisible. The little work by Howard Duncan on "Practical Curve Tracing" (Longmans) will be useful.

FIG. 27. MERCHISTON TOWER.

(Published by S. Hooper, 1790. From *The Merchistonian*, 1912-13.)

A word of caution may be allowed. Although an equation may express in one line what would require pages of verbal description, it must not be forgotten that it is, after all, but a kind of shorthand, and must never be permitted to serve in place of a clear conception of the process itself. The same thing may be said of structural formulæ in chemistry, which are only a very convenient way of expressing certain facts in the play of molecular forces, whose nature is as yet unknown. This fact sometimes seems to be in danger of being forgotten, and "bonds" regarded as actual material threads holding atoms together.

Structural formulæ sometimes say too much even when regarded merely as records of experimental results; in other ways they do not say enough. A. W. Stewart points out (*Chemical World*, December 1912, p. 415) that in the formula for acetic acid, if written thus:—

$$\begin{array}{c} CH_3\text{—}C\text{=}O \\ \;\;\;\;\;\;\;\;| \\ \;\;\;\;\;\;\;\;OH \end{array}$$

there is experimental evidence that the three methyl hydrogen atoms are different from the hydroxyl one, but that there is no evidence for the existence of a CO group; none of the reactions characteristic of its presence are given by acetic acid. In order to make the formula inform us of the difference between the various hydrogen atoms, which is not directly indicated, we have to treat the groups CH_3 and OH as wholes, saying that hydrogen is not the same when united with oxygen as when united with carbon. Moreover, carbonyl, as such, is not present in acetic acid; when CO is united with OH, a new radical, COOH (carboxyl), is formed, which must itself be taken as a whole, so that the formula of acetic acid is more correctly written:—

$$CH_3 - COOH.$$

These components of organic compounds behave, as it were, as elements, and, strictly speaking, to make structural formulæ more complete in certain ways, it would be necessary to give each of these radicals a distinctive symbol. The essence of chemical combination is, of course, that the properties of elements are changed when united with others, as in the common illustration of mercuric iodide. The object of these remarks is merely to advocate more critical use of structural formulæ than is apt to be made by a certain school of chemists, who appear to think that, if a formula can be made to indicate the possibility of a particular mode of combination, the fact is in itself proof that such a reaction actually occurs. G. H. Lewes (1864, p. 131) refers to the profound psychological mistake of holding "that whenever man can form clear ideas, not in themselves contradictory, these ideas must of necessity represent truths of nature." This view was, at one time, very widely held, and even by so great a man as Descartes. For further discussion see Karl Pearson's book (1911, chapter viii.).

THE CARBON ATOM

The question may properly be asked, What are the peculiarities that make organic chemistry a special domain and of especial importance in physiological science? The reason lies, as van't Hoff (1881, i. p. 34 ff., and ii. p. 240 ff.) points out, in the characteristic qualities of carbon itself. This author enumerates five items:—

1. The quadrivalence renders possible an enormous number of derivatives of any one compound.

2. The capacity of carbon atoms of uniting with each other allows a great variety of modes of combination.

3. Its position in the periodic system, in the middle between positive and negative elements, gives it the power of uniting with the most different elements—hydrogen, nitrogen, oxygen, chlorine, etc. (see the table in Nernst's book, 1911, p. 180). Owing to this, it is readily capable of alternate oxidation and reduction, and thus of acting as a carrier of energy.

4. When three of its valencies are saturated, the fourth valency has a "positive" or "negative" character, according to the nature of the groups in the other three places. Thus while—

$$\begin{array}{l} H_2 \diagdown \\ \;\;\;\;\;\;\;\;\;\; C\text{—} \\ NO_2 \diagup \end{array}$$

is usually "negative,"

$$H_3 \equiv C\text{—}$$

is markedly "positive," like hydrogen.

5. The slowness of reaction or inertia of the carbon compounds is of much significance in vital phenomena. As an illustration, methyl sulphonic acid is much more stable than sulphurous acid, having a methyl group in place of hydrogen.

EFFECT OF TEMPERATURE ON THE RATE OF REACTIONS

Chemical reactions arrive at their point of equilibrium and stop dead at it without overshooting. They are, in fact, aperiodic, like processes in general

taking place against resistance. This being so, a formula similar in form to that of Ohm's law in electricity must hold. Thus :—

$$\text{Velocity of reaction} = \frac{\text{chemical force}}{\text{chemical resistance}}.$$

Chemical force is a function of the free energy; very little is definitely known as to chemical resistance, except that it is greatly diminished by rise of temperature.

All chemical reactions are, therefore, increased in rate by rise of temperature. Some confusion is apt to arise with respect to endothermic reactions, on account of the effect of temperature on the equilibrium point, to be described presently. Endothermic reactions require to be supplied with energy from their surroundings, since the products have a greater store of potential energy than the bodies from which they are produced; but it must not be forgotten that they progress of themselves. A chemical reaction takes place, in fact, when the intensity factor of the energy associated with the original mixture is greater than that of the final system (see Mellor's book, 1904, p. 25), whether the reaction be endo- or exothermic.

From the standpoint of the kinetic theory of heat, it is easy to see why all processes conditioned by rate of molecular movement are accelerated by rise of temperature. But, as Nernst points out (1911, p. 680), it is not so easy to see why the acceleration of chemical reactions is as great as it is. A rise of 10° C. usually doubles or trebles this rate (Law of van't Hoff), whereas "the velocity of molecular movement in gases, and in all probability in liquids also, is proportional to the square root of the absolute temperature." So that, if it has a value of 100 at 20°, it will only increase to 101·7 at 30°, instead of to 200; Goldschmidt (1909, p. 206), however, has shown that only those molecules react whose velocity exceeds a certain high value, so that the difficulty disappears.

Conclusions are sometimes drawn as to the nature of a particular process from the value of the temperature coefficient. This quantity varies so much, not only according to the position on the scale of temperature at which the reaction happens to take place, but also in individual cases, that, on this ground alone, caution must be exercised.

For example, the saponification of ethyl butyrate by barium hydroxide between 50° and 60° has the low value for a chemical reaction of 1·33 for 10° (Trautz and Volkmann, 1908, p. 79), whereas diffusion, a physical process, has a value nearly as high, viz., 1·28 (Nernst, 1888, p. 624). Chick and Martin (1910, p. 415) find that the heat coagulation of hæmoglobin has the extraordinarily high temperature coefficient of 13·8 for 10°, while that of albumin is even higher. It is of interest that P. von Schroeder (1903, p. 88) finds that gelatine solution, in a particular condition, has a viscosity at 21° represented by 13·76, whereas at 31° it is only 1·42; that is about ten times less for 10° rise of temperature. As will be seen later, colloids of the type of gelatine play a large part in vital processes. The temperature coefficient of the rate of absorption of water by the seeds of barley has recently been shown by Adrian Brown and Worley (1912, pp. 546-553) to be of the order of that usually regarded as characteristic of chemical reactions. They also find that the rate is an exponential function of the temperature. This is, as Mellor points out (1904, p. 394), very rare for a physical process. The increase of the vapour pressure of a liquid is one of these rare cases, and, in fact, the value of the exponent in Brown and Worley's experiments is the same as that of the vapour pressure of water. The bearing of this fact on the effect of temperature on chemical reaction in general will be found in Chapter VIII.

The impossibility of forming conclusions as to the physical or chemical nature of a process from the temperature coefficient of its velocity is well shown by the work of Knowlton and Starling (1912, p. 206), on the effect of temperature on the rate of the heart-beat in the isolated heart-lung preparation. This rate is a linear function of the temperature, as shown by Fig. 28. In other words, a given rise of temperature produces the same increase at different points of the scale. But such a relationship is what we find in the simplest physical process, such as the expansion of a gas. Therefore, if the temperature coefficient is any index, the heart-beat is a purely physical process. This is obviously an absurd conclusion. We know that rise of temperature accelerates the chemical changes in the heart muscle, as evidenced by the increase in the oxygen consumption (Lovatt Evans, 1912, p. 231), and, in fact, it is very interesting to find that this increased

metabolism is directly proportional to the increase of rate, so that we have again a linear relation. It will be plain that, in such a case as that before us, one cannot speak of a "coefficient" in the strict sense. If such a number be calculated for any particular temperature, it will not apply to any other temperature.

Consider indeed, for a moment, the complexity and variety of the forms of energy change involved in a muscular contraction—surface and volume energy, thermal, electrical and chemical energy. I think that it must be admitted that to attempt to draw conclusions from the temperature coefficient of the entire process does not seem likely to lead to results of much value. This remark, of course, applies to the activities of living protoplasm in general, as well as to muscle.

Krogh (1914, 1), moreover, finds that the velocity of embryonic division in amphibia, fish, insects, and echinoderms cannot, even approximately, be expressed by the van't Hoff formula of temperature effect on chemical reactions. Between normal limits, the relation is a linear one. In a further paper (1814, 2), Krogh finds that there is no optimum temperature for the evolution of carbon dioxide, and that this process also follows a linear law.

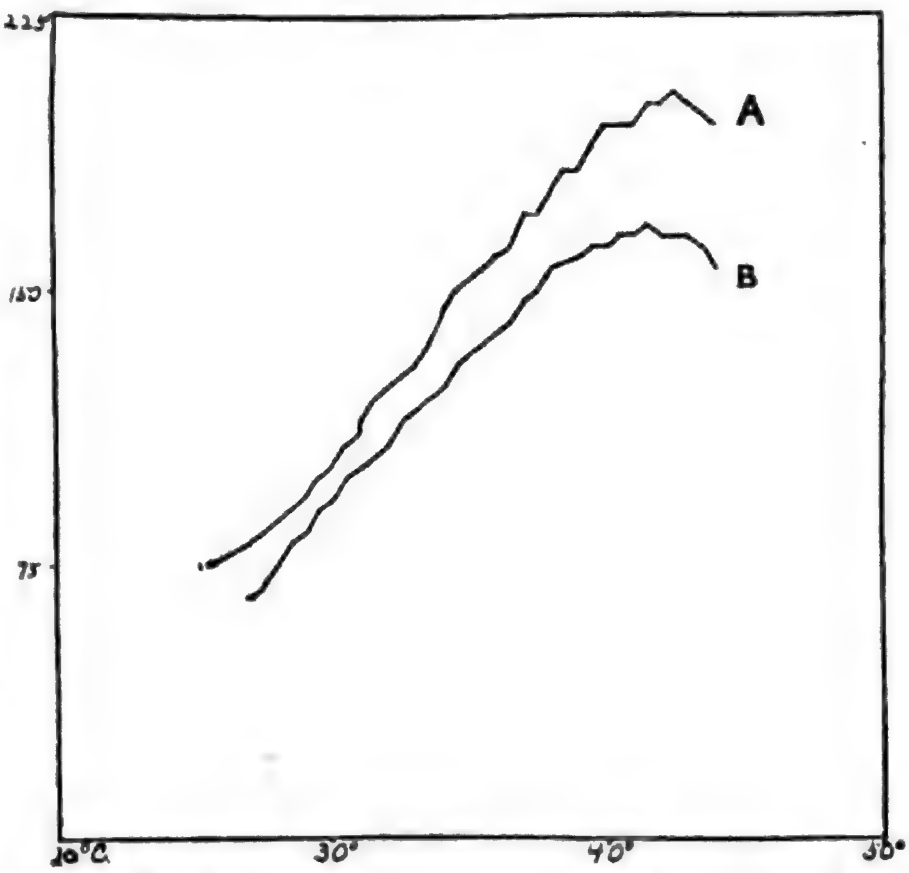

Fig. 28. Effect of Temperature on the Rate of the Beat of the Isolated Mammalian Heart.

Abscissæ—temperature.
Ordinates—number of beats per minute.

Between the limits of 26° and 40°, in which the heart continues to contract normally, the relation is linear. There is no temperature "coefficient."

(Knowlton and Starling, 1912, p. 217.)

Regarded from another point of view, we must remember that these vital phenomena are taking place in heterogeneous systems, that is, in systems consisting of various solid and liquid phases. When not coarsely heterogeneous, they are, at least, colloidal, or ultra-microscopically heterogeneous. We have, therefore, several processes in addition to the purely chemical one going on together, viz., diffusion of constituents of the reaction to and from the surface where the reaction occurs, similarly to the action of hydrochloric acid on a plate of marble, followed by condensation on the surface and so forth. As Nernst points out (1911, p. 587), the velocity of the process as a whole will be conditioned by that factor which takes place at the slowest rate. In many cases this is diffusion, as in the experiments of Brunner (1904, p. 56). But it does not seem necessary that this should always be the case. It is conceivable that the chemical factor in the complex may be slowed down, as by a low temperature, so far as to become slower than the diffusion factor. In such a case, the "limiting factor," to use Blackman's expression, would be transferred from the diffusion process to the chemical reaction. I am not aware, however, that any instance of such a change has been met with.

Further discussion of heterogeneous reactions will be found in Chapter X., when treating of catalytic action. In the present place, attention is directed mainly to the complexity of any given vital process, and to the uncertainty as to what factor is the controlling one in the velocity of the reaction, or which one it is whose temperature coefficient is being measured.

ANIMAL TEMPERATURE AND ITS REGULATION

From the preceding paragraph it will be obvious that, for rapidity of adaptation to outside changes, it is of advantage to the reacting organism that its processes be carried on at a raised temperature. Suppose, however, that a chemical reaction, such as an oxidation, is set in progress. Heat produced accelerates the reaction, and it will tend to become faster and faster, verging on an explosion. Some means of regulation of such reactions is clearly necessary. One obvious way of doing this, in the case of oxidation, is to limit the supply of oxygen. Organisms provided with circulation of blood conveying oxygen have the power of cutting down the supply to their various parts by methods to be described later. In warm-blooded animals the chief source by which the temperature is kept up is muscular contraction, controlled by the nervous system.

Apart from its effect on chemical reactions, a high temperature is also of advantage in its action on physical processes, diminishing the internal friction of liquids such as blood, hastening diffusion, and so on.

The question will be discussed further in Chapter XIV.

THE EFFECT OF TEMPERATURE ON EQUILIBRIUM

The confusion that is sometimes made between the effect of heat in increasing the rate of a change, and its effect on the position of equilibrium in a reversible reaction, has been already alluded to. We have seen that the rate of any reaction, exothermic or endothermic, is accelerated by rise of temperature. On the position of equilibrium, its effect may differ in individual cases, as may be seen theoretically from the consideration that, of the two balanced opposing reactions, either one may be accelerated more than the other. If, for example, the synthetic reaction in the case of alcohol, acid, ester, and water were accelerated more than the hydrolytic one, the equilibrium would be moved in such a direction that more ester would be present and less alcohol and acid, and conversely.

In actual fact, the effect in question differs *in direction* in the case of exothermic and endothermic reactions. The law expressing this relationship was deduced thermodynamically by van't Hoff (1884, pp. 161-176). For the reasoning adopted, the reader may consult Mellor's "Chemical Statics and Dynamics" (pp. 395-401). The "Principle of Mobile Equilibrium," introduced by van't Hoff, may be expressed briefly as follows: Any change of the temperature of a system in equilibrium is followed by a reverse thermal change within the system. By taking separately the three possible cases, the meaning will be made more intelligible.

1. Suppose that a reaction has taken place by which a substance B has been formed from another substance A. If this reaction has been accompanied by the *evolution of heat*, a rise of temperature will cause an *increase* in the quantity of A. In other words, the reaction is partially reversed. Heat is absorbed and the temperature falls. Since the law holds for physical as well as chemical phenomena, it may easily be remembered by consideration of the condensation of water vapour (A) to liquid (B), which is accompanied by evolution of heat. The law tells us that raising the temperature will increase the quantity of steam (A), as every one knows.

2. If the reaction is endothermic, accompanied by *absorption of heat*, rise of temperature will cause *decrease* in the quantity of A, that is, the reaction will go on further. One may say that, as the reaction requires heat to progress, an extra supply will help it on. Heat again is absorbed. An illustration, merely to assist the memory, is the case of ether (A). By evaporation spontaneously to vapour (B) it cools, and, if prevented from absorbing heat from its surroundings, it may become so cold that evaporation practically ceases. If heat be supplied, more vapour (B) will be formed, and the liquid phase (A) will diminish.

3. The third case is that of a reaction in which *no thermal change* occurs. Here a rise of temperature will have no effect on the relative amounts of A and B.

In a more general form the principle was given by Le Chatelier in 1884 (see 1888, p. 210). In this form, it can be seen how it is comprehended in the second law of energetics (see Lewis,

1916, vol. 2, p. 140). It may be stated thus:—When any influence or factor capable of changing the equilibrium of a system is altered, the system tends to change in such a way as to oppose and annul the alteration in this factor. If it be temperature, for example, the effect is to decrease the change in temperature.

An instructive case to consider in this connection is that of the taking up of a dye by a substance which is stained by it, say paper, or tissue in the process of histological staining. As will be seen in subsequent pages, this process is representative of many of those occurring in living cells. I found (1906, p. 187) that the amount of dye which a piece of paper of a certain size will take up from a given solution of Congo-red, if allowed to remain in it until no further amount is taken up, is *less* at 50° than at 10°. Now, whether this process is one of pure adsorption (=surface condensation) or also partly chemical, it is no doubt associated with the production of heat. Calling the system, paper in contact with dye solution, A, and the dyed paper, B, van't Hoff's law, the first case above, tells us that rise of temperature causes increase in A, as experiment shows.

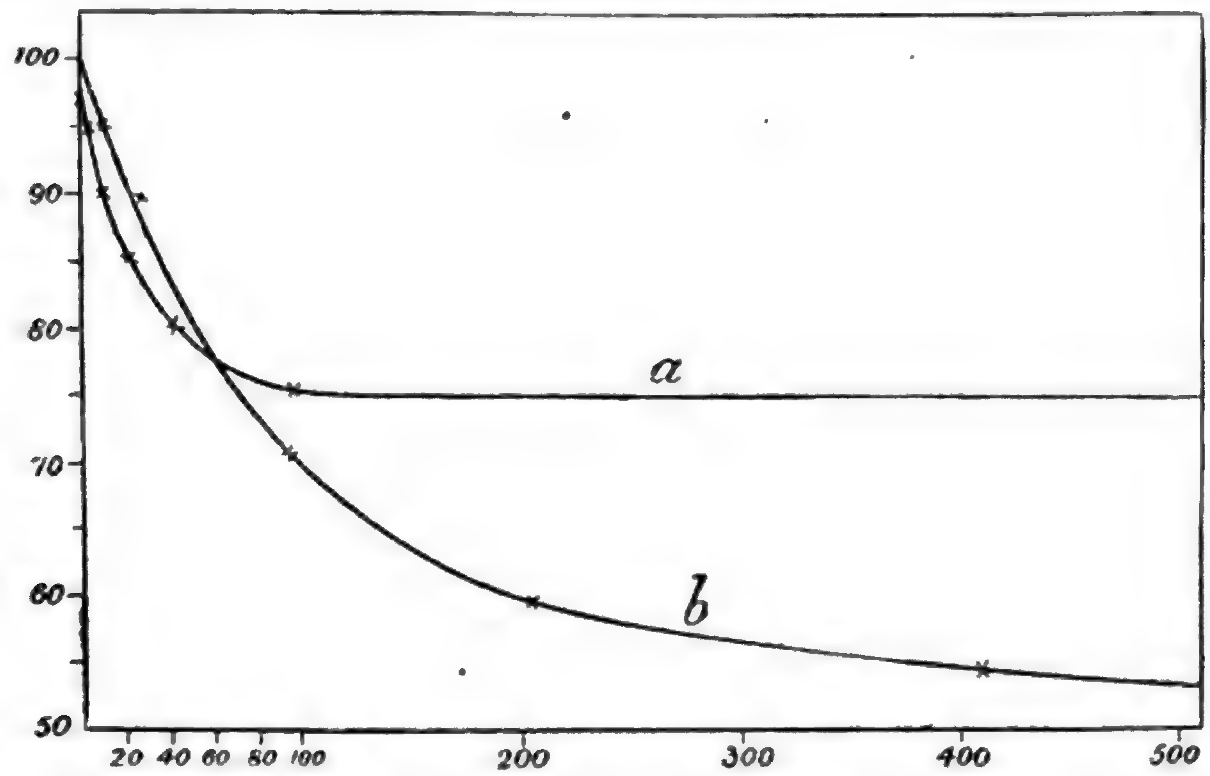

FIG. 29.—EFFECT OF TEMPERATURE ON THE RATE OF ADSORPTION OF CONGO-RED BY PAPER.

Abscissæ—time in minutes.
Ordinates—total amount unadsorbed at the time—

a, at 50°.
b, at 10°.

At the higher temperature, the *rate* of adsorption is faster, although the total amount adsorbed when equilibrium is reached is less.

(Bayliss, 1911, 1, p. 187.)

Although, however, at the higher temperature there is *less* product formed, yet the *rate* at which this is formed is *greater*. The curves of Fig. 29 serve to show this fact. It will be seen that, at the higher temperature, equilibrium was attained in about 100 minutes (curve *a*), whereas at the lower temperature (curve *b*), it was not quite complete at the end of the experiment (twenty-four hours). The amount taken up at the lower temperature was rather more than one half of that originally present in the solution; at the higher temperature, only one quarter. This experiment will be found (Chapter XXI.) to have some bearing on the way in which oxygen is carried by hæmoglobin.

The great influence that temperature has on both rate and equilibrium in chemical and physical processes necessitates care in the maintenance of a constant known temperature in investigating them. The means of doing this will be found in the textbooks dealing with practical physical chemistry, such as those of Findlay, Ostwald-Luther or Spencer.

SUMMARY

The essential characteristic of life is incessant change. To produce change, work must be done. The power of doing work is due to the possession of energy.

Of the two great laws dealing with energy, the first tells us that, while any one kind of energy may be transformed into any other kind, there is never any gain or loss.

The second law deals with the conditions under which these changes take place and the proportion of one kind that can be transformed into another.

Although the total energy cannot be altered, the amount of it available for conversion into other forms and capable of doing work, *i.e.*, the *free* energy, is not constant, and indeed, in the present state of the universe, so far as we are able to investigate it, free energy always tends to diminish. This fact, a matter of invariable experience, is known as the "Principle of Carnot and Clausius," and is of great importance in the interpretation of many physiological problems.

There are two factors which, multiplied together, give energy. One of these, of the nature of a "strength," is called the "intensity" factor; the other, of the nature of a space or mass, is called the "capacity" factor. As regards the latter factor, energy can be added algebraically, but not as regards the former.

In the animal body, energy is derived from chemical combination. This form of energy is readily converted into various other forms, without the necessity of passing through the form of heat.

In the vegetable organism, energy is derived ultimately from the sun's rays. It follows, therefore, that animal energy has the same origin.

The maximal work of a chemical process can be calculated by means of a formula due to Nernst; it depends on the position of equilibrium in the reaction considered as reversible, and is greater the nearer this position is to that of complete change in one direction.

That manifestation of molecular forces known as surface energy plays an important part in cell phenomena, owing to the large variations of which it is capable in a small space. This is due to the changes in its capacity factor, surface area, chiefly by aggregation of colloidal particles.

The phenomena peculiarly characteristic of vital changes are those associated with the actual process of transfer or transformation of energy. Many non-vital phenomena show also a special degree of activity in such states.

The total energy obtained from a food-stuff by complete oxidation, the "heat of combustion," does not of necessity imply that stuffs of the same heat of combustion are of equal value as sources of available energy. The distinction between free and bound energy must be taken into consideration. The "struggle for existence" is for the possession of free energy.

The formula for the work done in compressing a gas from a volume v_2 to v_1, or from pressure p_1 to p_2, viz.—

$$\mathrm{RT}\log_e\frac{v_2}{v_1} \text{ or } \mathrm{RT}\log_e\frac{p_1}{p_2},$$

is also applicable to that done in concentrating a solution from one osmotic pressure to another, to the potential of metallic electrodes, and to the case of certain solutes confined by a membrane permeable to one ion only, to mention cases of physiological interest.

The properties of the carbon atom make it of especial value in the transformation of chemical energy, so that the body of doctrine known as organic chemistry is of fundamental importance in physiology.

The effect of a rise of temperature on the rate of chemical reaction must be

carefully distinguished from that on the position of equilibrium. The former is always increased, while the latter is controlled by van't Hoff's "Principle of Mobile Equilibrium." Whether it is changed in the direction of further progress of a reaction, or the reverse, depends on whether the reaction is accompanied by evolution of heat or the contrary. In the former case, a rise of temperature throws the reaction back, while the opposite is the case in the latter. If the reaction is thermo-neutral, no change is produced by alteration of temperature.

The temperature coefficient of complex processes in heterogeneous systems, such as those of living cells, cannot be used to indicate whether such a process is chemical or physical in nature.

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W. Ostwald (1912, 2).
W. C. McC. Lewis (1916, vol. 2, pp. 1-74).

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Helm (1894).

Philosophical and Practical Applications.

Ostwald (1912, p. 1), "Der energetische Imperativ," i.

The following essays of Boltzmann (1905) will be found of interest:—

3. "Der zweite Hauptsatz der mechanischen Wärmetheorie" (pp. 25-50).
9. "Zur Energetik" (pp. 137-140).
8. "Ein Wort der Mathematik an die Energetik" (pp. 104-127).

The works of Willard Gibbs can only be attacked with profit by the expert mathematician.

CHAPTER III

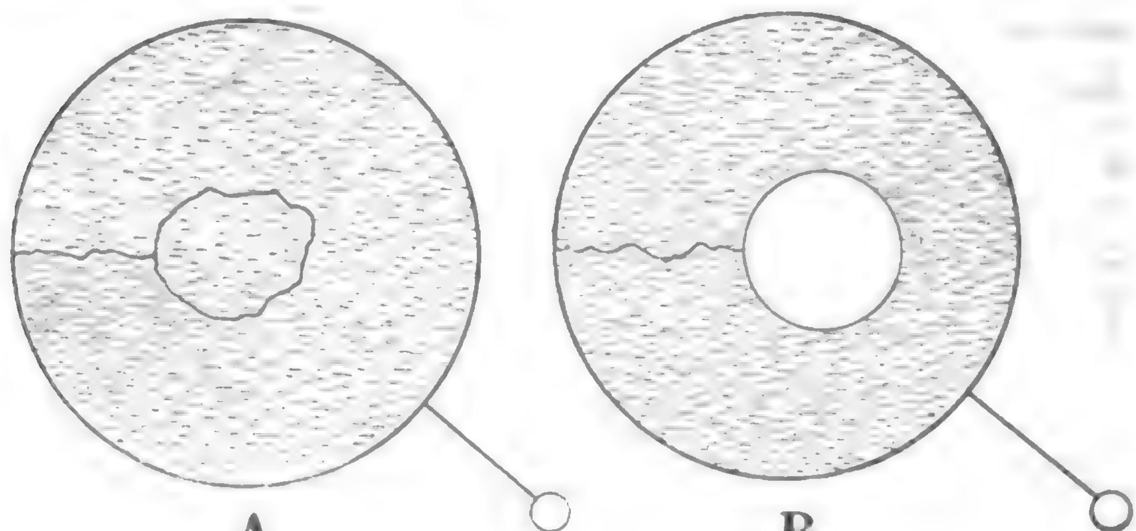
SURFACE ACTION

It has been shown in Chapter I. how living cells are made up of a highly complex system of constituents, not mixing together—liquids, solids, and sometimes gases. Some of the solid substances, the "hydrophile" colloids, contain water in such proportion that many of their properties approximate to those of liquids.

Investigation has made it plain that where these different "phases," as we have been taught to call them by Willard Gibbs, come into contact with each other at their interfaces, the properties are not the same as in the main mass.

SURFACE TENSION

One of the most obvious phenomena of this kind is that shown by the surface of contact of liquids with gases, solids, or other liquids immiscible with them. This surface behaves as if stretched.



A **B**

FIG. 30. RING OF IRON WIRE, ENCLOSING A SOAP FILM.

In *A* there is a loop of fine silk floating in the film.
In *B* the portion of the film inside the loop has been broken by touching it with a pointed bit of filter paper. The result is that the tension of the film between the ring and the loop causes this film to contract as much as possible, thus drawing the loop into a circle, the figure of maximum area.

(Van der Mensbrugghe.)

One of the simplest ways to demonstrate this is due to van der Mensbrugghe (1866, p. 312). A loop of fine silk is taken and tied to a wire ring. If the whole be dipped into soap solution, so as to produce a film, the loop floats in the film; the silk thread forming its boundary is quite loose, and can be readily moved into any shape by means of a fine needle wetted with the soap solution (see Fig. 30). The film inside the loop is now broken by touching it with a bit of filter paper cut to a fine point. The loop is immediately drawn to a circular form by the tension of the film surrounding it, and can be felt to resist attempts to change its shape by the needle. The soap solution should be prepared by the method of Boys (1912, p. 170) from pure sodium oleate, with the addition of about 25 per cent. of glycerol.

The best way of showing that the form taken by a liquid when free is that with the least surface, namely the sphere, is by the use of ortho-toluidine, as described by Darling (1911). This liquid has the same density as water at 22°, but, since it has a higher coefficient of expansion, it is less dense above 22° and more dense below that temperature. If a beaker half full of water at 22° is taken, and a solution of sodium chloride of about 0·3 per cent. is run in

at the bottom, so as to form a lower stratum of slightly higher specific gravity, ortho-toluidine can be run in at the junction of the two liquids by means of a tap-funnel, and spheres of 6 to 8 centimetres in diameter can be made.

It is interesting to note that the phenomena shown by such suspended spheres of liquid were chiefly investigated by Plateau, the physicist of Ghent, after he became blind owing to gazing at the midday sun for experiments on vision. His researches were published in 1873. In his work he was assisted by his son-in-law, van der Mensbrugghe, whose name we have already met with.

A method of measurement of surface tension is by the use of Searle's apparatus, made by Pye, of Cambridge (Fig. 31). The pull of the tension of a liquid film is made to twist a wire of phosphor-bronze by a known amount, which is compared with that effected by a known weight. A rectangular glass microscope slide is clipped to one end of the lever, which also carries a scale pan. The counterpoise is then adjusted so that the lever is horizontal when the lower edge of the slide is just immersed in the liquid. The reading on the scale is noted and the liquid removed. The lever will rise considerably. After drying the glass slide, the lever is brought down to its previous position on the scale by adding weights to the scale pan; in other words, a force is applied to twist the wire to the same extent as the surface tension of the liquid did. If A is the length of the slide in centimetres and T its thickness, the total length of the film is $2(A+T)$, since both sides of the slide are active. Then M being the mass in grammes added to the scale pan, its weight is 981M in dynes, and the surface tension in dynes per centimetre is

$$\frac{981 \times M}{2(A+T)}.$$

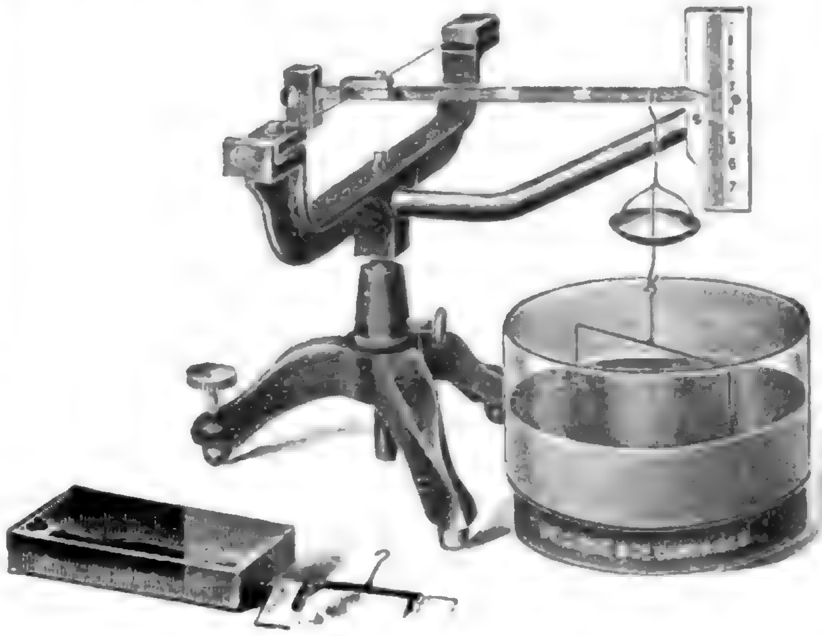


FIG. 31. SEARLE'S TORSION BALANCE FOR MEASURING SURFACE TENSION OF LIQUIDS.
(As made by Pye & Co., Cambridge.)

If it be wished to obtain a measurement of the absolute surface tension at a water-air interface, it is best to use tap water, since this is less likely than distilled water is to contain greasy matter, which has a powerful effect in lowering surface tension, as we shall see later. With Searle's apparatus I have found no difficulty in a lecture experiment in obtaining readings of 71·6 dynes, or 98 per cent. of the correct value, 73. The weight needed in an actual experiment to produce the same torsion of the wire as the pull of the water did was 1·11 grams, a sufficiently obvious weight.

The effect of surface tension in regulating the size of drops falling from an orifice is also used as a method of measuring the surface tension of liquids. It is sometimes called the "*stalagmometer*" method, and is due to Quincke. The size of a drop will increase until its weight balances the tension of its surface film, which is holding it up against gravity. As soon as this size is exceeded the drop will fall. In practice, the number of drops in a known volume of the liquid is counted, and this number is, obviously, inversely proportional to the size of the drops, and this again is proportional to the surface tension—the larger the drop the greater the surface tension. Account must be taken of the weight of the drop, that is, the specific gravity of the liquid must be known. On the assumption that the upper boundary of the liquid is a plane surface (but see Spencer, 1911, 1, p. 147):—

$$\frac{\text{Number of drops of water} \times \text{density of liquid}}{\text{Number of drops of liquid}} \times 73 \text{ dynes per cm.}$$

Another method is founded on the rise or fall of the level of a liquid in a

capillary tube, according to whether it wets the glass or not. This change of level is due to the curved shape of the meniscus or surface separating liquid from air, so that the surface tension has a vertical component which pulls up the liquid against gravity, or presses it down, according to whether the meniscus is concave or convex. The best form of apparatus for this method is that of Röntgen and Schneider (1886, p. 203), especially in the modification described by Schryver (1910, p. 109).

A means of rendering this measurement more accurate was pointed out to me by W. A. Osborne. Two capillaries of different but known diameters are taken, and the difference of heights to which the two liquids to be compared rise in the two capillaries is measured. By this device the measurement of the height of the meniscus from the body of the liquid is unnecessary, a somewhat difficult and uncertain one. Since the total height in each case is inversely proportional to the diameter of the capillary, and directly proportional to the surface tension, the difference of the heights of the two liquids is also so proportional. We know the diameters of the two capillaries and the surface tension of one liquid, so that it is easy to calculate that of the other. This method is also recommended by Michaelis and Rona (1909, p. 496).

The formula for rise in capillary tube is:—

$$\frac{\text{Height} \times \text{radius of tube} \times \text{density} \times 981}{2}.$$

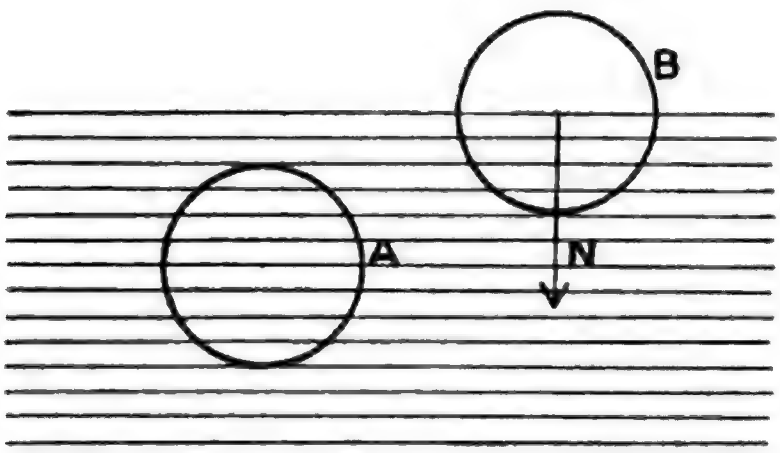


Fig. 32. Diagram to illustrate the origin of surface tension from internal pressure of liquids.

The molecule *A* is exposed to equal attractive forces on all sides. The molecule *B*, at the surface of the liquid, on the other hand, is exposed to unbalanced forces, of which the resultant is a pressure in the direction of *N*. Equilibrium will result when the number of molecules at the surface is the least possible; that is, the surface area tends to a minimum.

(Errera, 1907, p. 16.)

The precise cause of the existence of surface tension is too complex for discussion here. Briefly, one may say that it is due to the forces of attraction between the molecules of a liquid, producing what is known as the "internal pressure" of Laplace (1845, iv. p. 389). This pressure can be calculated, and amounts to several thousand atmospheres (Stefan, *Wied. Ann.*, **29**, p. 655). The molecules in the body of the liquid are exposed to these forces equally on all sides. Those at the surface are exposed to unbalanced forces tending to draw them in (see Fig. 32). The result of this is that the surface of a liquid is always the least possible, or, in other words, is pulling itself together. One may see the necessity of a minimum surface also from the point of view of energetics. Since there are forces drawing the molecules inwards, work is required to bring them to the surface, therefore the greater the surface, the greater the energy contained in it; but, as we have seen, free energy always tends to a minimum. For further details see Freundlich (1909, pp. 6-14). The explanation of the properties of the free surface, by regarding it as the seat of tension, is due to Thomas Young (1805, p. 82), who speaks of unbalanced molecular cohesive forces at the surface as the cause of the tension.

The values of the surface tension of pure liquids vary greatly. The following numbers in dynes per centimetre will serve to illustrate this:—

Water - - - -	73
Alcohol - - - -	22
Ether - - - -	16

Since the surface tension is not altered by enlarging the surface, as in blowing a soap-bubble, it follows that the pressure inside a small bubble is

greater than that inside a large bubble, contrary to what happens when an india rubber ball is blown out. The state of affairs in a soap-bubble is due to the greater curvature of the small bubble, so that the component producing internal pressure is greater in the smaller one. Fig. 33 shows this in a diagram. The two curved lines are of the same length. The vertical component, that is, the line drawn vertically perpendicular to the chord of the arc, is obviously greater in the arc with the greater curvature.

The fact just mentioned indicates the possibility of great pressure being produced in very minute spheres of liquids, such as we find in certain colloidal solutions.

SURFACE ENERGY

If the surface of a liquid is in a state of tension, it is clear that work may be done by it, when the surface is able to diminish. Surface tension, in fact, is the intensity factor of a kind of energy whose capacity factor is the area of surface, thus:—

Surface energy = surface tension × surface area.

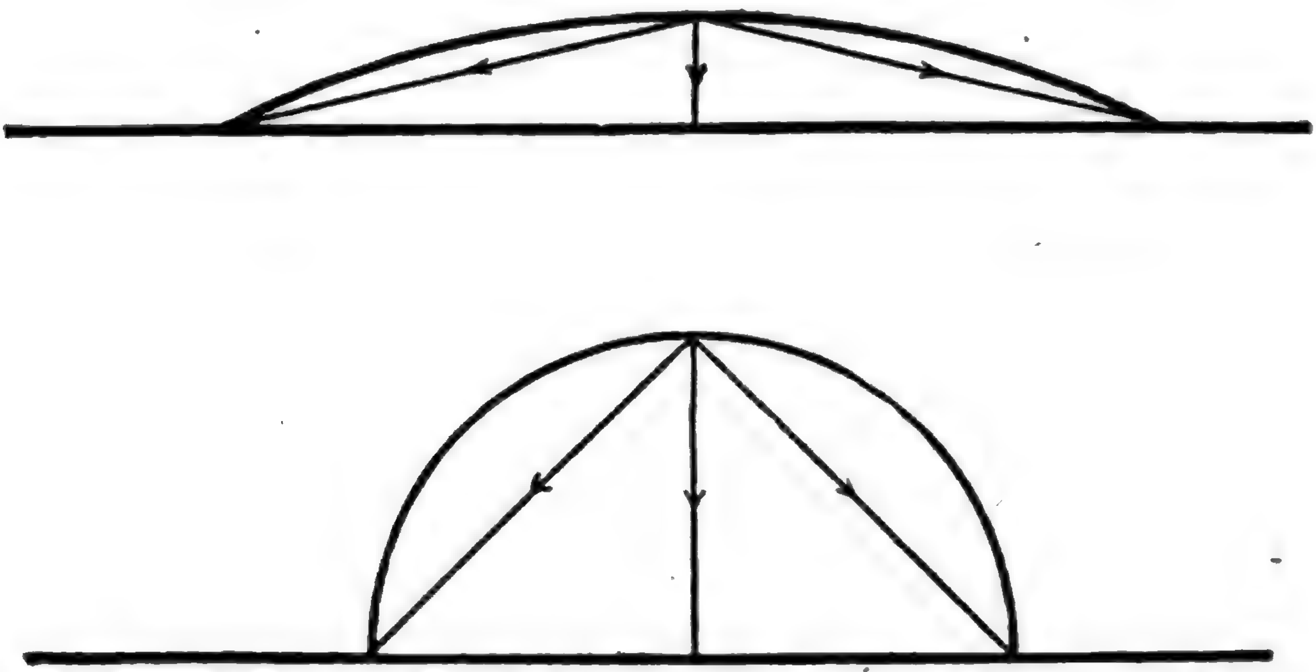


FIG. 33. DIAGRAM TO ILLUSTRATE THE EFFECT OF CURVATURE OF THE SURFACE ON THE PRESSURE INSIDE A SOAP BUBBLE, A DROP OF LIQUID, OR A SOLID PARTICLE.

The length of the arc, and therefore the total amount of surface tension, is the same in both figures. The internal component of the surface tension may be roughly represented by the length of the vertical line in each case.

What is the source of this energy? There can be little doubt that it is ultimately chemical. The fact that it differs according to the chemical constitution of the liquid is sufficient to show this. Hardy (1912, p. 621) has recently made some important experiments on this question. Various liquids, insoluble in water, spread out in a thin film when dropped on its surface, owing to the fact that they lower the surface tension. Substances of great chemical stability, such as the heavy liquid hydrocarbons, refuse to spread at all, and only very slightly lower the surface tension. Esters, glycerides, for example, produce great fall of surface tension and spread widely. The suggestion is made that this effect is due to decomposition at the interface, causing contact difference of potential between film and water.

SURFACE TENSION AT VARIOUS INTERFACES

Hitherto we have confined our attention to the interface between various pure liquids and air. When two immiscible liquids are in contact, there is also a state of tension at the interface, but less than that when either is in contact with air. It can be measured by the drop method, the stalagmometer

being filled with the heavier liquid, and having its orifice immersed in the lighter one. Of course, proper correction must be made for the effective weight of the drops in the liquid, compared with that in air.

Since the surface energy at the contact of two liquids is less than the sum of that between each of them and air, it follows that when two liquids, previously in contact with air, are brought into contact with each other, work is obtained. An important fact found by Hardy (1913) is that this work is greatest in the case of the most chemically active fluids, such as esters, alcohols, and acids; smallest in the case of the saturated hydrocarbons. The merest trace of oleic acid, added to an inactive hydrocarbon, reduces its surface energy to an enormous extent.

Interfaces between liquids and between these and solids are met with in physiology more frequently than those between gases and liquids.

As regards the surface tension at the interface between *solid and liquid*, we have, unfortunately, no direct method of determination, but Ostwald (1900, ii. p. 503) indicated an indirect one, depending on the greater solubility of small particles than of large ones. This fact is due to the action of molecular forces at the interface, causing the liquid component to have greater solvent power. The larger the total area of surface on the particles, the greater will their solubility appear to be. This is the reason why large crystals grow at the expense of small ones, since the solution which is saturated as regards the large particles or crystals is not saturated with respect to the smaller ones (see also the book by Freundlich, 1909, pp. 143-145).

W. J. Jones (1913) has made renewed measurements by the method referred to, and finds that the surface tension of barium sulphate, in contact with its saturated solution, is 1,300 dynes per centimetre. It will be noted that this is a very high value compared with that between liquid and liquid, or liquid and gas. At the water-air interface, for example, the surface tension is only 75 dynes. The fact is of importance in connection with the large degree of adsorption manifested by the surfaces of solids, such as charcoal, as will be seen later.

As a rule, substances in solution in liquids lower the surface tension at the interface between these liquids and air. Inorganic salts (such as sodium chloride) raise it, but not to any great extent. There are great differences between the actions of different substances in their action on surface tension. Some, bile salts, for example, have a very great effect. The same statement applies to the interface between liquid and liquid, except that it appears that all bodies in solution, even inorganic salts, *lower* the surface tension.

W. C. M'C. Lewis (1909, 1, p. 469) finds that inorganic salts lower the interfacial tension between a hydrocarbon oil and water. He also calls attention (1910, 1, p. 632) to the circumstance that if we take into account the curvature of the surface, and the densities of the two phases, we obtain a quantity, which may be called the "specific capillary constant," and that this constant is always lowered by dissolved substances, even when air is one of the phases.

A point to be remembered is that small amounts of dissolved substances produce, for equal amounts, a greater lowering of surface tension than larger amounts. The curve expressing the relationship is one of the family of parabolas (Freundlich, 1909, p. 65). The importance of this will be seen when we are discussing adsorption.

When living protoplasm is in contact with any solution, there must be surface tension at the interface. Some experiments by Kisch (1912, p. 152) are of interest here. Yeast and other fungi were found to be permanently injured as soon as the surface tension of the solution in which they were immersed became, by the addition of various substances, less than half of that between water and air. The actual concentrations required were:—

Ethyl alcohol - - - -	28 per cent.
Isoamyl alcohol - - - -	2 ,,
Acetone - - - -	30 ,,

The cells of higher plants were found by Czapek ("Ueber eine Methode zur direkten Bestimmung der Oberflächenspannung der Plasmahaut von Pflanzen-

zellen," Jena, 1911) to be more sensitive, being injured when the surface tension was reduced only to 0·68 of that between water and air. These results are difficult of interpretation, especially in view of the complex series of phenomena to be described presently under the head of adsorption.

ELECTRIC CHARGE

In addition to the surface tension produced by unbalanced molecular forces, there are various other ways in which the properties of substances at their boundaries with other phases differ from those in the main body of the substances. We have first to consider the electric charge. In any charged body, as we know from Faraday's researches, the charge is accumulated at the surface.

It is somewhat remarkable to find that the boundary surface between liquid and solid, or between immiscible liquids, is nearly always the seat of electrical forces. It has also been shown by Hardy and Harvey (1911, p. 220) that the interface between water and air is similarly the seat of an electric charge. The origin of this charge is not, in all cases, clear. Electrolytic dissociation at the surface will account for the existence and the sign of the charge in perhaps the majority of cases. In other cases, however, ionisation of this kind seems to be out of the question. Drops of petroleum in water have a negative charge, investigated by W. M'C. Lewis (1909, ii. p. 211), and those of aniline have also a negative charge (Ridsdale Ellis, 1912, p. 346). If the charge in this latter case were due to ionisation, it should be positive. Aniline, as a base, dissociates to a certain extent into OH' ions, which pass into the water, leaving the aniline ion with a positive charge. The same process must be supposed to occur at the surface of a drop suspended in water: the mobile OH' ions will travel off, leaving the heavy insoluble anions aggregated on the surface of the drops, which then behave as huge electro-positive ions. This explanation is quite satisfactory for particles such as those of aluminium hydroxide, which have a positive charge, but it does not hold for aniline. W. M'C. Lewis (1910, ii. p. 64) suggests an electronic origin for such cases, on the ground of the similar values (0·04 volt) found for very different chemical substances, suspensions, emulsions, and filter plugs. Burton (1906) has also shown that the same value is obtained for suspensions in methyl or ethyl alcohol or ethyl malonate. The question cannot as yet be regarded as completely solved. The work of Rudge (1914) on the electrification of dust is of interest in this connection.

The Helmholtz "double-layer" demands a word at this point, although an adequate treatment is impossible. Those interested should consult the paper in his "Gesammelte Abhandl.," i. p. 925; an account of the theory will be found in Freundlich's chapter v., "Die kapillarelektrischen Erscheinungen" (1909, pp. 184-262). It is unnecessary to remind the reader that an electric charge of a particular sign cannot exist without the simultaneous presence in its proximity of an equal and opposite one. The charge on the surface of a solid in a liquid, therefore, implies the existence of an equal and opposite one on the liquid side of the interface. This fact adds complexity to the interpretation of the phenomena now under consideration, but cannot be left out of account.

In later pages we shall see how the charge on a surface can be increased, diminished, annulled or reversed in sign by the presence of ions in the liquid which is in contact with it.

The effect of an electric charge on the mechanical surface tension is to reduce it. The elements of the surface, when they have charges of the same sign, mutually repel one another, so that the area of the surface tends to *increase*, in opposition to the effect of the surface tension to *decrease* it. The bearing of this fact on the stability of emulsions will be seen in the following chapter.

INFLUENCE ON SOLUBILITY AND ON CHEMICAL REACTION

The solubility of certain bodies is found to be different in the surface layer from what it is in the body of the liquid. For example, it was found by J. J. Thomson (1888, p. 254) that potassium sulphate is 60 per cent. more soluble

in the surface film. In the same work it is shown dynamically that surface tension will have a large effect in changing the degree of chemical combination (pp. 234-237).

Christoff (1912, p. 456) finds that the less is the surface tension of a liquid, the greater is the solubility of gases in the liquid. The values of the absorption coefficients (volume of gas dissolved by unit volume of liquid) at 0° of gases of interest to the physiologist are as follows:—

	Water.	Alcohol.	Ether.
Hydrogen	0·02148	0·06925	0·1115
Nitrogen	0·02348	0·12634	0·2580
Carbon monoxide	0·03537	0·20443	0·3618
Oxygen	0·04890	0·28397	0·4235
Carbon dioxide	1·713	4·3295	7·330

The values for water are those of Winkler; for alcohol, those of Bunsen; and for ether, those of Christoff.

The results obtained by Vernon (1907) are of interest here. He showed that oxygen is 4·5 times more soluble in oil and fat than in water, while nitrogen is 5·3 times more soluble. In a rough experiment which I made, it was found that carbon dioxide was rather more soluble in thick paraffin oil than in water. These various facts serve to show the futility of attempting to preserve solutions from the action of gases in the atmosphere by covering them with oil or hydrocarbons. They are also of importance in the results of exposure of animals to compressed air.

When gases are taken up by charcoal, it is clear that a large amount of compression must occur; some observers hold that, in the case of certain gases, there must be actual liquefaction. Heat must be evolved in this process, a fact whose meaning will be apparent later.

Some *chemical reactions* are accelerated at interfaces, others retarded. Thus, Freundlich (1906, p. 85) found an acceleration of the following reactions on the surface of charcoal: oxidation of formic, citric and mandelic acids, and of glycerol, hydrolysis of chlorine, esterification of alcohol with organic acids, decomposition of phenyl-thio-urea. Perman and Greaves (1908, p. 366) found that the rate of decomposition of ozone by heat depends on the extent of surface to which the gas is exposed and that in all probability the reaction takes place only there.

An interesting case of retardation of a reaction by surface forces is that called by its discoverer, Liebreich (1886), the "*dead space.*" This observer noticed that if a molar solution of sodium carbonate be mixed with a half-molar solution of chloral hydrate in a test tube, the turbidity, which gradually forms by the production and separation of chloroform, is absent from the surface layer of the fluid, and he was able to show that this clear space was really due to the reaction not having taken place therein. This retardation can be accounted for, thermodynamically, if the reaction resulted in an increase of surface energy, since all processes which lead to an increase of free energy are opposed. It is interesting to find, therefore, that it was found by Dr Monckman in the Cavendish Laboratory at Cambridge, that the surface tension increased considerably as the reaction went on (J. J. Thomson, 1888, p. 237). This effect is, no doubt, due to the comparative insolubility of chloroform and the disappearance of the chloral hydrate, from which it is formed.

ADSORPTION

Any substance dissolved in water lowers the surface tension at the interface between the solution and a solid, or immiscible liquid. With the exception of certain inorganic salts, this is also the case at the interface between the solution and a gas. Further, at these interfaces there is a local accumulation of free surface energy, which can be altered in amount by the deposition of substances at the interface. It follows, then, from the second law of energetics,

that dissolved substances which lower surface tension will be concentrated in this situation, on account of the fact that free energy will be lessened thereby.

This result is of fundamental importance, and was arrived at by Willard Gibbs (1906, i. p. 56) from thermodynamic considerations in 1878, and by J. J. Thomson in 1888 (1888, pp. 191, 192) from the dynamical point of view. It will be referred to in subsequent pages as the "Gibbs" or "Gibbs-Thomson" principle. It is really a particular application of the general doctrine of decrease of free energy, as shown by the headlines chosen by Gibbs himself for his work on "Heterogeneous Equilibrium," viz., the formulation by Clausius of the two laws of energetics, as given on p. 28 of the present volume. As applied to surface *energy*, the Gibbs principle has a wider application than may appear from the above statement of it as referring to surface tension. It may be expressed thus: Any process that diminishes the free energy at an interface will tend to take place, whatever be the nature of the energy concerned, whether mechanical, electrical, chemical, or other. If the surface has an electric charge, a process diminishing it will be favoured. If it possesses chemical energy, a reaction reducing this energy will take place, if possible; and so on.

Accordingly, any substance in solution in a liquid, in contact with the surface of another phase, will be concentrated on that surface, if, by doing so, the free energy present there is decreased. This process is called "*adsorption.*" Its characteristic is the relation to *surfaces* of contact. Whatever further process may follow it, chemical reaction, or diffusion into the body of the other phase, the first thing to take place is the local concentration. The rate at which subsequent events happen will naturally depend, by mass-action, on the amount of this condensation. Given the diminution of surface energy, the adsorption process is thermodynamically bound to take place, and any other explanation of the phenomenon is superfluous.

As an example, the well-known effect of charcoal in decolorising or clarifying a solution may be given. If a dilute solution of a dye, such as "night-blue," be mixed with charcoal, it can be almost completely decolorised. That the dye is not destroyed, or chemically combined with the carbon, can easily be shown by filtering off the latter, and extracting it with alcohol, which will be found to become of a deep blue colour. The process is, in fact, reversible.

In the case of the ordinary form of surface energy, Gibbs has given a formula by which the amount of dissolved substance concentrated at the interface can be calculated. Thus: Let Γ be the excess of solute in the surface layer above that in the body of the solution, C the concentration of the solute, R the gas constant, T the absolute temperature, and σ the surface tension at the interface. Then $\frac{d\sigma}{dC}$ represents the change of surface tension with change of concentration of solute, which can be measured, and

$$-\Gamma = \frac{C}{RT} \cdot \frac{d\sigma}{dC}.$$

The way in which this equation is obtained is beyond the scope of this work. The appearance of R and T is due to the assumption that dilute solutions obey the gas law, so that the formula cannot be of general application.

This formula has been tested experimentally by W. C. M'C. Lewis and by Donnan and Barker, and found to give values in accordance with experiment in cases where the conditions are such that no complication due to other forms of surface energy, especially electrical, intervene. Lewis (1909, i. p. 486) found in the case of caffeine on the surface of petroleum, and (1910, iii. p. 136) of aniline on the surface of mercury, satisfactory agreement with the values calculated from the Gibbs formula. Donnan and Barker (1911, p. 573) obtained similar results in the cases of nonylic (pelargonic) acid, and of saponin at the interface between water and air. Nonylic acid has an extraordinarily high capacity of lowering surface tension.

Condensation of substances at the interface between their solutions and air shows itself in an interesting way in the experiments of Ramsden (1904). Certain substances, of which a list will be found in the original paper, such as white of egg, saponin, and quinine, are actually deposited in a solid form, so that the surface film of the solution becomes rigid.

One of the simplest ways to see this fact is to blow a bubble with a solution of saponin, say 1 per cent., as a soap-bubble would be blown. If air be then sucked back out of the bubble, or it be allowed to contract spontaneously, collapse is even and regular in the case of the soap-bubble, so that it remains spherical. In the case of saponin, on the contrary, the film has ceased to be elastic, and can only collapse by falling into folds. It is sometimes possible to see little rods of the solid in the film. A similar phenomenon is found to take place with egg albumin, and is said to be the reason why cooks find that a froth beaten up for méringues, if allowed to stand, cannot be made again into a froth; the albumin, in fact, has gone out of solution by surface coagulation. This coagulation in surface films is also, no doubt, the cause of the inactivation of enzymes when shaken with air, as found by Schmidt-Nielsen (1909 and 1910).

When surface tension is measured, as in the experiments of several workers, by means of vibrating drops or surface waves, the surface tension plays the part of elasticity in the ordinary form of wave motion in air, so that, when this surface tension changes, the rate of vibration changes also. When pure liquids are investigated by this dynamic method, in which the surface is being continually renewed, the same values are obtained as by static methods, such as rise in a capillary tube or drop method, where time is allowed for the surface to attain a state of equilibrium. With *solutions* of substances which lower surface tension, on the other hand, and are therefore concentrated in the surface layer, it depends upon the rate at which this adsorption takes place whether the two kinds of method give identical values. Conversely, if the values are not identical, it is clear that the adsorption has not had sufficient time for completion before a new surface is formed in the dynamic method. To take a well marked instance: A ·025 per cent. solution of sodium oleate has a static surface tension of 26 dynes, but a dynamic one of 79 dynes, practically the same as water, so that no adsorption has taken place in the time allowed before a new surface is formed. The fact is of interest in that it shows that the actual process of adsorption is not instantaneous, although it is extremely rapid.

The Gibbs principle implies, as will be obvious, that if a substance *raises* surface energy, its concentration at an interface will be *lowered*, giving rise to *negative adsorption*. Such a case has been described by Lagergren (1898). When sodium chloride solution is shaken with charcoal, its concentration is raised, owing to its being displaced from the interface and sent into the main body of the solution. This effect seems to depend on change in solubility with pressure, since the water film on the surface of the adsorbing powder is, probably, in a highly compressed state owing to molecular forces (Nernst, 1911, p. 124).

Although the principle of Carnot and Clausius shows that adsorption must take place if free energy is lowered thereby, we have, as yet, made no reference to the *forces* which produce this surface action. Titoff points out (1910, p. 674) that the quantity adsorbed in the case of gases increases with the well-known quantity a of the Van der Waals equation—

$$\left(p+\frac{a}{v^2}\right)(v-b)=\mathrm{RT}.$$

The meaning of this equation will be discussed later (page 149), but it may be stated here that a expresses the mutual attraction of the molecules. Therefore, as Arrhenius puts it (1912, p. 40): "The forces which produce adsorption are of the same order and of the same nature as those which cause the mutual attraction of molecules." This view is confirmed by the fact, shown by Freundlich (1909, p. 154), that the extent to which a series of different substances is adsorbed by charcoal follows the same order, although different in absolute amounts, when adsorbed by wool, silk, cotton, and so on.

Four special cases of adsorption are of interest to the physiologist, on account of the part they play in the phenomena with which he has to deal. These may be given here as illustrating the nature of the process. Other cases will appear in the course of this book.

I. *The Adsorption of Gases by Solids.*—This is familiar to all chemists in the use of charcoal. It is characteristic of adsorption to be diminished by rise of temperature, and here it is of importance to remember that this statement refers only to the condition of *equilibrium*, and that the *rate* of adsorption is increased by

rise of temperature, in accordance with the general rule. At a temperature of liquid air, charcoal adsorbs gases to such a degree that it is used by Dewar to produce a high vacuum.

According to Arrhenius (1912, p. 29) adsorption by charcoal of gases which liquefy with difficulty, such as hydrogen and helium, is directly proportional to their pressure at ordinary temperatures, and of all gases (as well as some dissolved substances) at high temperatures. The fact suggests that deviations may be due to something like liquefaction on the surface. Titoff (1910, p. 673), indeed, concludes that ammonia is partially liquefied, because of the rate of increase of heat of adsorption as 0° is approached.

Since the gas is condensed on the surface, according to some observers even liquefied in certain cases, and when gases are compressed, heat is evolved, it is not surprising to find that adsorption is attended by production of heat. Titoff (1910, p. 658, etc.) has determined this in a number of instances, and his data will be made use of later in discussing the nature of oxyhæmoglobin. It is scarcely necessary to remind the reader that this adsorption of gases by surfaces is not a chemical reaction. If oxygen combined with the charcoal used to make a vacuum, so that CO or CO_2 were produced, it is obvious that no disappearance of gas would take place. Moreover, the adsorbed gas can be driven off again by heat.

The adsorption of water vapour on the surface of vessels which have been dried in a vacuum desiccator is a well-known source of trial to the chemist.

When finely divided platinum is exposed to a mixture of oxygen and hydrogen, combination takes place between these gases, with the formation of water. Faraday (1834, p. 165) suggested as an explanation of this phenomenon that a condensation of the gases took place on the surface of the platinum, so that the molecules were brought into close contact.

It is interesting to note the clear conception of surface condensation which Faraday had formed. On p. 180 of his "Experimental Researches on Electricity" (1839) he speaks of an "attractive force of bodies" causing association more or less close, without at the same time producing chemical combination, but "which occasionally leads, under very favourable circumstances, to the combination of bodies simultaneously subjected to this attraction." On p. 181 he refers to "the attraction between glass and air, well known to barometer makers," and to the fact that they have no power of combination with each other. On p. 181, again, mention is made of the power of water vapour to condense *upon*, although not to combine with clay, charcoal, and turf, "assisted a little, perhaps, by a very slight solvent action" in the latter case. (See the present author's letter to *Nature*, vol. xciv. (1914) p. 253.)

The question will come up for further discussion in Chapter X.

II. *The Adsorption of Sugar.*—With respect to the mechanism of the action of enzymes, it is of importance to know whether sugars and related substances are adsorbed. It appears that sugar does not lower the surface tension at the interface between water and another phase to any great extent. It has been shown, however, by Michaelis and Rona (1909, p. 492), and by Parkin (1911, p. 16), that adsorption does occur. The diminution of surface energy must, therefore, concern one or more of the other forms of surface energy which we have referred to. Michaelis and Rona, in fact, suggest that the adsorption may be due to the change of compressibility or of solubility at the interface (see also Wiegner, 1911, p. 126).

III. *Salts.*—Inorganic salts, although raising surface tension at the air-water interface, lower it at a water-hydrocarbon interface, as Lewis has shown (1909, i. p. 469). Theoretically, then, there is a possibility of adsorption at such an interface. The actual fact can be demonstrated experimentally. J. J. Thomson (1888, p. 192) describes an experiment by Dr Monckman and himself, in which a deep coloured solution of potassium permanganate emerged almost colourless after trickling through finely divided silica. Samec (1911, p. 155) quotes an investigation by Kugel, in which it was found that the apparent solubility of the more insoluble salts might be as much as one thousand times more in starch solutions than in water, owing to adsorption by the starch granules.

IV. *The Nature of Dyeing and Staining.*—The first stage of this process is almost certainly one of adsorption. How far other processes, such as solid solution and chemical reaction, play a part in later stages, will be discussed presently.

ELECTRICAL ADSORPTION

The adsorption of electrolytes and of dyes is a more complex process than that of mere reduction of surface tension, since electrical forces come into play.

In the experiments of Lewis, caffeine and aniline, and in those of Donnan and Barker, nonylic acid and saponin, obey the Gibbs formula. These, it will be noted, are all practically non-electrolytes. Lewis found, on the contrary, that bile-salts and dyestuffs, such as methyl orange and Congo-red, were taken up in much larger amount than the Gibbs formula would indicate. These latter compounds, however, are electrolytes, *i.e.*, they are dissociated in water, with the formation of electrically charged products. The non-dissociated part, moreover, tends to form aggregates of a colloidal nature, which carry charges.

We have already seen how most insoluble surfaces immersed in water have a negative charge, some few a positive one. The origin of this charge does not concern us here, and will be treated in future pages. The point to be noted is that it gives rise to a considerable amount of free energy on the surface. If, therefore, the deposition of any substance, from solution in the water, upon such a surface would reduce the electrical potential there, it will, by the Gibbs principle, tend to take place. Suppose that the surface is that of charcoal, which has in water a negative charge, and that to the water we add substances with positive charges, such as colloidal ferric hydroxide, or a salt which dissociates with production of positive and negative ions. The colloid or the cation of the salt will be deposited on the surface, so that its charge is neutralised.

Perrin (1905, p. 100) was the first to suggest that electrical forces might play a part in the phenomena of dyeing, and V. Henri and Larguier des Bancels (1905) called in the aid of such forces to explain the fact that aniline blue, an electro negative colloid, is taken up by gelatine, itself an electro-negative colloid, in very small amount, because of the mutual repulsion of their charges. If, however, barium nitrate, which dissociates with formation of positively charged barium ions, be added, these ions discharge the dye particles (from Perrin's work, it is more probable that it is the surface of the gelatine that is discharged), so that there is no longer repulsion, and the gelatine becomes deeply stained. The first systematic investigation of this electrical adsorption was made by myself (1906). I found that the adsorption of various electrically charged bodies by electrically charged surfaces depended on the sign and the amount of the respective charges. An electro-negative surface, say that of filter paper, will take up large quantities of an electro-positive substance, such as night-blue, but only a trace of a negative dye, such as Congo-red. The amount adsorbed also depends on the amount of the charge, as is indicated by its connection with the dielectric constants of the constituents of the system; for example, more Congo-red is taken up from dilute alcohol than from water. The charge of paper is proportional to the difference between its dielectric constant and that of the liquid in which it is immersed. Paper itself has a dielectric constant of 2·82, water one of about 80, pure alcohol one of 26 (see the article by Graetz in Winkelmann's "Physik," 2te Aufl., Bd. IV., pp. 112, 144, and 137). Hence the negative charge of paper is lower in alcohol, and a negative dye is more readily adsorbed.

I found further, that when neutral salts, having no chemical action on the materials concerned, such as sodium chloride in the cases of Congo-red and night-blue, are added, the effect is to *increase* the adsorption of *negative* dyes and to *diminish* that of *positive* dyes. The explanation will be obvious; the positive ion (Na) of the salt diminishes the negative charge of the paper, in accordance with the Gibbs principle, and consequently the adsorption of a similarly charged body is facilitated while that of an oppositely charged one is retarded. The adsorption of colloidal arsenious sulphide (electro-negative) was found to be affected in the same way as that of Congo-red. Addition of a trace of gelatine or albumin to the solution prevents the effect of electrolytes, a phenomenon whose explanation will be found when we come to discuss the colloidal state.

Similar theories with regard to dyes, but less complete, since the actions of added salts and of dielectric constants were not included, were subsequently put forward by Pelet-Jolivet (1910), Michaelis (1908), and by Gee and Harrison (1910).

That the electric charge on surfaces is in reality diminished, neutralised, or even reversed by ions with charges of opposite sign, has been shown experimentally by Perrin (1904). The method used was to determine the rate at which water passes through diaphragms of paper or other substance, which had been exposed to the action of various electrolytes, in obedience to the attraction or repulsion of charged electrodes at opposite sides of the diaphragm. If this latter, for example, is negatively charged, the water in contact with it will be positively charged, and therefore attracted by the negatively charged electrode (anode).

Emil Baur (1913) describes a method of demonstrating and measuring the change of potential at a lipoid-water interface when anion or cation is adsorbed thereon. A model, on this principle, of the electrical organ of the fish is also described. The change of electromotive force produced in this manner is permanent and always of the sign predicted by the hypothesis, so that the effect appears to be actually due to adsorption (see Chapter XXII.).

Acids and alkalies are very active in this power of conferring electric charges on surfaces, no doubt owing to the great mobility of $H^{\cdot}$ and OH' ions, responsible for the effect. Graphite, for example, can in this way be made positive. Lachs and Michaelis have shown (1911, p. 5) that when such electro-positive graphite is immersed in a solution of potassium chloride, the negative ion (Cl') is adsorbed, while electro-negative graphite adsorbs, in preference, the positive ion ($K^{\cdot}$).

It is, however, incorrect to say, as these authors do, that the Gibbs principle fails in such cases. If the statement of this principle is understood to refer only to mechanical surface energy, it is true that electrical energy is left out of consideration; but this is clearly not the intention of Gibbs himself, who would make it apply to all forms of surface energy. In fact, it is really a deduction from the principle of Carnot and Clausius, which controls all forms of energy whatever.

From the point of view of energetics, we may formulate the main fact of electrical adsorption as follows. Any process that will reduce the electrical energy at a surface will tend to take place. Hence, for example, if a surface has a negative charge, positively charged bodies will be concentrated upon it, so as to annul its charge. These bodies may be positive ions (cations) or colloidal aggregates. It is not clear, however, from this point of view alone, why the charge is, in many cases, not merely reduced to zero but actually reversed in sign (Perrin, 1904, p. 640). According to Harrison (1911, p. 20) the negative electrical charge on "diamine-blue" is annulled by aluminium sulphate in low concentration, but, in greater concentration, converted into a positive one. It is probable that, although the electrical energy at the surface, in such cases of reversal of sign, is greater than it is at the stage in which the original charge is abolished, other forms of surface energy, such as the mechanical one due to surface tension, may be decreased. The question of adsorption of ions which decrease surface tension has been considered by Freundlich (1909, p. 245), Elissafoff (1912), and Ishizaka (1913). The last observer finds that, in the precipitation of aluminium hydroxide, a strongly adsorbed (organic) anion, such as that of salicylic acid, is more powerful than one which is weakly adsorbed, such as a univalent inorganic anion, or that of sulphanilic acid. We see thus the possibility of a charge being increased, if the ion conferring the charge is one that is strongly adsorbed, owing to its effect in diminishing the mechanical surface energy. Similarly, Freundlich and Schucht (1913, p. 646) find that, in the precipitation of a negative colloid by cations, those of the heavy metals and of organic bases are more active than would be expected from their valency, and that this is to be accounted for by the fact of their great mechanical adsorption.

CHEMICAL ADSORPTION

The doctrine of energetics, as applied to chemical reactions, teaches us that such reactions will be favoured at interfaces if they lower the chemical potential there. The condition required for such cases is, of course, that the chemical nature of the phase regarded as that one at whose surface the reaction occurs is such as to be capable of reaction with the substance in solution. The difference between such surface phenomena and reactions in true solution is that in the latter case the law of mass action is strictly obeyed, the active mass present being equivalent to the number of molecules of the solute; whereas, in the former case,

the extent of surface, or the number of molecules situated there, is the controlling factor, corresponding to the active mass. The surface of the same quantity of matter may vary enormously, according to the degree of subdivision, as already pointed out. The subdivision may indeed be carried out in imagination so far that molecular dimensions are reached, in which case ordinary chemical action is being dealt with. This possibility of the existence of every intermediate stage is apparent, and it is, perhaps, this fact that has led to many of the loose statements made by some writers on adsorption. Although theoretically, chemical adsorption, as defined above, should be included under the general name, it is usually understood that the more physical forms, due to changes in surface tension or electrical charge, are meant when adsorption is spoken of as distinct from chemical combination.

In cases of "*specific*" *adsorption*, where a surface takes up preferentially a particular substance, it appears that the chemical nature of the surface must be taken into account. At the same time, when we remember the manifold possibilities of differences in surface tension, electric charge, etc., it seems unlikely that recourse need be had to chemical phenomena, except in rare cases (see van Bemmelen, 1910, pp. 423-430; Freundlich, 1909, pp. 153-162; Barger and W. W. Starling, 1915). The physical properties of a substance depend on its chemical constitution.

Some examples may be given :—Wöhler and Plüddemann (1908, p. 664) found that carbon and red oxide of iron adsorb benzoic acid ten times as strongly as they do acetic acid. Chromium oxide adsorbs both acids equally; while platinum black adsorbs acetic acid slightly more than benzoic acid, but neither to any great extent. These apparently specific adsorptions can scarcely be of a chemical nature. Another case which may have a bearing on the question of specific adsorption is given by Marc (1913, p. 692). Crystalline substances, such as barium carbonate, only adsorb crystalloids when these are isomorphous, or crystallise in a similar form to that of barium carbonate. They are supposed to be able to form a solid solution on the surface of the adsorbent. Thus, potassium nitrate is adsorbed, since it, like barium carbonate, belongs to the rhombic system. Sodium nitrate, of the hexagonal system, is not notably adsorbed. Calcium carbonate, of the hexagonal system, on the other hand, adsorbs sodium nitrate, but not potassium nitrate. Since calcium carbonate can be obtained also in crystals of the rhombic system, it seems possible to test the hypothesis; these crystals should adsorb potassium nitrate but not sodium nitrate. It must be remembered also that potassium nitrate can crystallise in the hexagonal system, isomorphous with sodium nitrate. This deposition of a salt on an isomorphous crystal might be supposed to be merely the ordinary growth of a crystal in a solution of an isomorphous salt, say, calc-spar increasing in size by the addition of layers of sodium nitrate; but, as it appears to follow a complex parabolic law, surface concentration, according to the laws of adsorption, needs taking account of.

Freundlich (1909, p. 514) points out that gelatine only adsorbs sugar after having been treated with formaldehyde. We have seen above that there is a considerable difference in the structure of gelatine after the action of formaldehyde, as shown by Hardy (1899, i. p. 165). But we must also remember that formaldehyde combines chemically with proteins, so that the interpretation of this fact is not quite simple.

Drury's work (1914) shows that the condensation of a solute on to a surface is markedly influenced by the *previous treatment* of, or by the gas condensed on, that surface.

The physical configuration of the surface may also play a part when both adsorbent and body adsorbed have surfaces of a definite structure. A rough illustration may explain what is meant. A flat surface and one covered with projecting points cannot get into close contact, whereas two flat surfaces can do so. This idea is at present, however, purely hypothetical. The problem of specific adsorption has not yet received adequate investigation.

COMBINED EFFECTS

The various forms of surface energy may be present at the same time on the same surface, and it is of some interest to know how they affect one another. The action of an electrical charge on mechanical surface tension is to diminish it, as may be seen from the following consideration. Surface tension is due to the mutual attraction of the elements of the surface; when these elements receive an electric charge, they repel one another, being of the same sign, and thus a force is present in an opposite direction to that of surface tension.

It appears that electrical adsorption exceeds in amount that due to diminution of surface tension, so far as the cases at present known indicate. We see in the experiments of Lewis with sodium oleate (1909, p. 494) that the amount adsorbed by a water-oil interface was one hundred times greater than that calculated from the Gibbs formula to be due to diminution of surface tension.

VELOCITY OF ADSORPTION

There is every reason to suppose that, when a substance reaches the surface at which it is adsorbed, the actual process of attachment itself is of very great rapidity. The difference between static and dynamic surface tension, referred to on p. 56 above, shows, however, that the rate of surface concentration is not absolutely instantaneous. Although this is the case, it is clear that, when an obvious interval of time is observed to elapse in an adsorption experiment before equilibrium is attained, in many cases several hours, what is being measured is the time taken for the substance to diffuse from the more distant parts of the solution to the adsorbing surface. As would be expected, it is found that the time taken for attainment of equilibrium is shortened by shaking (Arendt, 1915).

EFFECT OF TEMPERATURE ON ADSORPTION

The effect of temperature on the *rate* of adsorption, in accordance with the previous paragraph, is found to be of the same order as that which it has on diffusion processes. In the case of Congo-red and filter paper, my experiments (1906, p. 188) showed the coefficient to be 1·36 for 10° C. Brunner (1904, p. 62) found that for the diffusion of benzoic acid to be 1·5.

Although the *rate* of adsorption is *increased* by rise of temperature, in agreement with the usual rule, the *amount* adsorbed when equilibrium is reached is *diminished*. Heat dissociates an adsorption compound. This fact is familiar in the case of charcoal, where the gas adsorbed at a low temperature is given off again on heating. In the case of Congo-red, my experiments showed that the amount taken up was in inverse linear proportion to the temperature (1906, p. 190). When the temperature was raised to 100° C., the dye was fixed in the paper and could not be removed by washing. Chemical combination appears to take place, and also goes on very slowly at ordinary temperatures. This fact will be referred to again below.

The decrease of adsorption by rise of temperature is, no doubt, to be explained by the fact that surface energy itself is anomalous in having a *negative* temperature coefficient. The surface tension of a particular sample of tap water was found to be 73·8 dynes at 17° and 65 dynes at 60°. Lactic acid in 44 per cent. solution at 18° has a value of 50·5 dynes, and of 47 dynes at 67°. In accordance with these data, it was found that 2 grams of charcoal at 0° adsorbed 51 per cent. of the lactic acid from 20 c.c. of 0·71 per cent. solution, but only 42 per cent. at 40°. If we consider the surface tension at the interface between a liquid and its vapour, we see that it must vanish at the critical temperature, since the boundary surface disappears. Hence, we should expect that the surface tension would decrease as the temperature rises towards this critical point.

The physiological significance of the fact may be illustrated by the case of muscular contraction, whose strength is diminished by rise of temperature. Weizsäcker (1914) has shown experimentally that one of the components of the process has itself a negative temperature coefficient. The conclusion may be drawn that surface energy plays an important part in muscular contraction.

HEAT OF ADSORPTION

Since adsorption is decreased by rise of temperature, the van't Hoff principle of mobile equilibrium implies that it takes place with evolution of heat. This is easy to detect in the case of gases, as we have seen; in that of liquids and solids it is difficult to distinguish it from the heat of liquefaction or of dilution, etc.

THE ADSORPTION FORMULA

One of the most characteristic properties of an adsorption process is that the amount taken up is not in direct linear relationship to the concentration of the adsorbed substance in the solution in equilibrium with the surface. Suppose a is the amount adsorbed from a certain solution, then the amount adsorbed from a solution of twice the concentration will not be $a \times 2$, but

$a \times$ *some root of* 2, or less than twice; this root, expressed as exponent $\left(\frac{1}{n}\right)$, usually lies between the values of 0·1 and 0·5. In the latter case it is, of course, the simple relation of the square root, but, as we shall have many opportunities of seeing in succeeding pages, it is very rarely that it is precisely of this value. A table of values for a number of typical cases will be found on pp. 150 and 151 of Freundlich's work (1909). In other words, the more dilute the solution, the greater is the proportion of its contents that is adsorbed. The equation expressing this relationship is given by Freundlich (1909, p. 146) in the following form:—

$$\frac{x}{m} = a.C^{\frac{1}{n}}$$

where x is the amount adsorbed by the surface m, from a solution whose *final* concentration is C, a and $\frac{1}{n}$ being constants for a particular surface and solution. The temperature is supposed constant, so that the expression is that of the adsorption isotherm. a may be defined as the quantity adsorbed by unit surface from a solution which is of unit concentration when in equilibrium with the amount adsorbed by the surface. Its value varies considerably in different instances, according to surface tension, electric charge, and so on. The range of its values is very much greater than that of $\frac{1}{n}$.

The relation of this formula to that correlating diminution of surface tension with concentration, as given on p. 52 above, will be evident. If we consider the effect of successive deposits on a surface, it will be clear that the first one will cause greater diminution of surface energy than succeeding ones, and each of these less than its predecessor. Each successive deposit occurs on a surface whose energy is already lessened by the previous deposit. Finally, a state of saturation is reached.

The curve expressed by Freundlich's equation is usually, but incorrectly, called an "exponential" one. Properly speaking, an exponential curve is one whose equation has one of the *variables* as an exponent: $y = a.e.^{bx}$. Our curve is one of the forms of the general equation to the family of parabolas: $y = ax^n$; when $n = 2$, or $\frac{1}{n} = 0·5$, the curve is the ordinary parabola, when $n = 3$, it is called a cubic parabola.

In order to determine the values of $\frac{1}{n}$ and a for a series of experimental results, the simplest way is to plot out the values on logarithmic paper. Freundlich's formula may be written thus, by taking logarithms throughout:—

$$\log \frac{x}{m} = \log a + \frac{1}{n} \log C.$$

This formula is that of a straight line inclined to the axes. If the values of $\log \frac{x}{m}$ be represented as ordinates, and those of log C as abscissæ, $\frac{1}{n}$ is the tangent of the angle made by the straight line joining the series of points with the axis of abscissæ. This line cuts the axis of ordinates at a point above the origin; the distance of this point from the origin is the value of log a.

Although this formula satisfies adsorption processes through a wide range, it has been shown by G. C. Schmidt (1911, p. 660) that a more complex one is needed to satisfy extremes of concentration, and he gives the following:—

$$\left(\frac{a-x}{v}\right) S = Kxe^{\frac{A(S-x)}{S}}$$

where x is the amount adsorbed, a the amount of substance originally present, and v the volume in which a was dissolved. $\frac{a-x}{v}$ is then the concentration of the solution in equilibrium. S is the amount at the maximum, *i.e.*, the amount adsorbed when in equilibrium with a saturated solution, and, therefore, $\frac{S-x}{S}$ is the proportion of the amount adsorbed at a given concentration to

that adsorbed at saturation. A and K are constants. In the case of acetic acid and charcoal, this formula gives correct values for all concentrations of acid between 1 and 3,000.

The reason for taking saturation into account is that a surface already completely covered cannot take up any more substance, since no change of surface energy would result. This fact is found to be in agreement with experimental data.

In Schmidt's equation the constant S expresses the maximum amount adsorbed in saturation, and A refers to the amount adsorbed at a particular concentration. Now, Arrhenius points out (1912, p. 31) that the product of S and A in Schmidt's experiments is, within the limits of experimental error, equal to the reciprocal of $\log_e 10$ or 0·4343. If this is so, Schmidt's equation amounts to the integral of the following differential equation:—

$$\frac{da}{dc} = \frac{1}{K} \times \frac{S - a}{a},$$

where c is the concentration. This represents, in a simple form, how the amount adsorbed in different concentrations is inversely proportional to the amount already adsorbed (a), and directly proportional to the distance from the point of saturation ($S - a$). Arrhenius finds that the phenomena of adsorption follow very closely this formula, except in the cases where the amount adsorbed is very small, on account of the large value of the heat of adsorption for the first quantities adsorbed (Arrhenius, 1912, p. 37). Titoff (1910, p. 659) finds for nitrogen the heat of adsorption per cubic centimetre of adsorbed gas, for the first small amounts, 0·373 gram-calorie, and when nearly saturated, 0·203 gram-calorie. For small values of a, in fact, the isotherms giving $\log a$ as a function of $\log p$ (= concentration), instead of being straight lines, diverge until they cut the axes of co-ordinates at 45°, thus obeying the law of Henry.

If a process is found experimentally to be best expressed by parabolic formulæ of the kind given above, the conclusion must not be drawn hastily that it is an adsorption. Other facts must be taken into consideration. For instance, suppose a substance is soluble in two immiscible solvents in contact with one another, but to a greater degree in one than in the other, it will be distributed in a certain ratio between the two, this ratio being known as the *partition coefficient*. If the dissolved substance is in single molecules in both solvents, as succinic acid in ether and water, a simple linear relationship holds, whatever the concentration. But, if the substance is associated in one of the solvents, so that the number of the molecules is halved or otherwise diminished, as in the case of benzoic acid, which is bimolecular in benzene, the ratio is no longer a linear one, but an exponential one, *e.g.*, in the case of benzoic acid in water and benzene, the concentration in water is equal to the square root of the concentration in benzene (Nernst, 1911, pp. 495-498). We see that the concentration of a substance in one phase may vary as a *power* of that in the other phase. If we find, then, that n in the Freundlich formula works out in a particular case to be a whole number, say 2, it might be a simple case of partition between two solvents, in one of which the substance is bimolecular. It is obvious that no difficulty arises when the exponent is such as to be an impossible one, except as an adsorption. Such is the case when it would imply the existence of fractions of molecules in one of the solvents. In the case of the adsorption of arsenious acid by freshly precipitated ferric hydroxide, as investigated by Biltz, the exponent is one-fifth. As Nernst points out (1911, p. 499), if this were a case of distribution between solvents, arsenious acid must have a molecular weight in ferric hydroxide one-fifth of that which it has in water. But in water it is already in single molecules. Again, as is pointed out by Philip (1910, p. 227), the concentration of carbon dioxide on charcoal increases proportionally to the cube root of the pressure in the experiments of Travers (1907). If this were a case of solution in charcoal, the carbon dioxide must have a molecular weight in the charcoal one-third of that in the gaseous state, which is not possible. The gas is evidently condensed on the surface.

Arrhenius ("Medd. k. Vetenskaps akad. Nobelinstitut," [2], No 7, 1910, quoted by Marc, 1913) has proposed a simple formula, to apply to the adsorption of gases by charcoal. It is pointed out that the compressibility of gases obeys the same formula; adsorption is regarded, accordingly, as a purely molecular property of the adsorbed matter and not as a surface phenomenon. It appears, however, from the work of Marc (1913) that the formula of

Arrhenius applies only to a very limited number of cases of adsorption, so that it is probable that the fact of the satisfactory application of this theory to certain cases is due to the connection of surface tension with molecular attraction, in accordance with the Young-Laplace theory. The actual process of adsorption in any particular case is a complex of several factors.

Langmuir (1916, 1918) treats the properties of surfaces from a chemical point of view. The molecules in the surface layer are supposed to arrange themselves in such a way that the residual affinities are drawn inwards. Chemical action being due to the electro-magnetic field around atoms, surface energy is a measure of the potential energy of the stray field extending outwards, and the molecules arrange themselves so that this stray field is the least possible. Other atoms or molecules can then be united to certain atoms in the surface, which may thus be completely covered or saturated, or only partially so. For further details see Lewis (1918, pp. 461, etc.).

ADSORPTION COMPOUNDS OR COLLOIDAL COMPLEXES

If we take a (colloidal) solution of the free acid of Congo-red, which has a blue colour, and add to it, quickly, a solution (also colloidal) of thorium hydroxide, a precipitate of a *blue* colour is formed. This precipitate can be filtered off, or better, centrifuged off, and resuspended in water. On allowing it to stand at room temperature, it slowly becomes red and part of it goes into solution; this change can be produced quickly by boiling. What is the explanation of this phenomenon?

The surfaces of the particles of the Congo-red acid have a negative charge, as can easily be shown by the behaviour to charged electrodes. The particles of the thorium hydroxide, on the other hand, have a positive charge. By aggregation together of these two substances the charges neutralise one another and free energy disappears, so that such a process will occur. But chemical combination only takes place very slowly, owing probably to very slight degree of ionisation of these two colloids. We have, in fact, free acid and free base in close apposition, but uncombined, as shown by the blue colour, which is that of the free acid. When chemically combined, the salts have a red colour, such as appears on heating the adsorption compound, or slowly at ordinary temperature. There are certain precautions to be observed to ensure success in this experiment, for which the reader is referred to my paper (1911, i. p. 83).

This peculiar type of compound is commonly met with where colloidal bodies are present, as in living organisms. It is rarely, however, that the nature of the complex is as clear as in the case given. Other properties must usually be taken into consideration. One of these is the absence of any quantitative, stoichiometric, relation between the constituents of the compound; they may be present in any ratio whatever. The colloidal complex of ferric chloride and ferric hydroxide, present in dialysed solutions of ferric chloride, may contain any percentage of chlorine from 65·5 (that of the chloride itself) through all stages to 6·4 per cent.

It will perhaps assist the reader to realise the distinction between chemical combination and adsorption if a few actual cases are considered briefly.

When a given quantity of charcoal is in equilibrium with solutions of acetic acid of varying concentrations, for each concentration there is a definite amount present in *both* phases, that is, there is always more or less acetic acid left in solution, however small the amount originally present. In seeking for a true chemical reaction to compare with this, it must be remembered that the acetic acid adsorbed on the surface of the charcoal is, for the time, fixed there; it is not in solution. The adsorption compound is similar to a precipitate. Our chemical reaction must therefore result in the production of a precipitate. Take, then, silver nitrate, and add to it varying percentages of sodium chloride. What happens is familiar to every one. At all concentrations of sodium chloride less than that equimolar with the silver present, *all* the chlorine is carried down and *none* is left in solution; at all concentrations of sodium chloride greater than equimolar, the amount of precipitate is always the same, whatever the concentration of the sodium chloride. The graph, instead of being parabolic, like that of adsorption, consists of two straight lines at right angles to one another. The figure by Freundlich (1909, p. 287) shows this in the case of the combination between diphenylamine and picric acid as investigated by Appleyard and Walker (*Journ. Chem. Soc.*, **69**,

1334, 1896). It can also be deduced theoretically from the law of mass action, as shown by Freundlich in the place referred to. Cases of combination between weak acids and bases which do not result in precipitation are not comparable.

When charcoal adsorbs either bromine, benzoic acid, aniline, or phenol, the value of the exponent in the formula of Freundlich varies only between 0·5 and 0·2. It is difficult to believe that any process of a chemical nature can be in question here.

The amount of any particular adsorption compound formed depends on the concentration, not the total mass of the adsorbed substance. Now, Brailsford Robertson (*Koll. Zeits.*, 3, p. 54) argues that this is also found in cases of reversible reactions like that of the formation of acetic ester from ethyl alcohol and acetic acid. He forgets, however, that the ester formed, although varying in amount with the concentration of the acid present, has always the same composition, whereas an adsorption compound would contain more acetic acid the greater the concentration of it in the mixture.

Raehlmann (1906, p. 152) has described how the constituents of certain adsorption compounds can be seen to be merely in close apposition. One of his experiments is as follows· The extract of a yellow wood, used in dyeing, and known as fustic, shows itself under the ultra-microscope to be a suspension of minute particles too small to be visible as separate dots. By the addition of alum, these "amicrons" can be caused to aggregate together to form larger ones, visible as such, and of a greenish colour. Serum albumin behaves similarly, and, under the influence of alum, forms yellow particles. The dye, "Congo fast blue," even without alum, consists of visible particles of a red colour by the reflected light of the ultra-microscope. Taking each separately, we have then green, yellow, and red particles. When the three solutions are mixed, an adsorption compound, which gives a green solution, is formed. This solution, under the ultra-microscope, is seen to consist of compound particles, each containing three of the simpler ones, one each of the red, green, and yellow ones. Fig. 34 is a diagrammatic representation of Raehlmann's fig. 4, the original being in colours. If albumin, Congo-blue, and fustic are mixed, without alum, the particles do not run together. It appears that Congo-blue, and probably also the other colloids, have a negative charge, which must be neutralised by the trivalent aluminium ion before aggregation can occur. The meaning of this experiment will be appreciated better after Chapter IV., "On the Colloidal State," has been read.

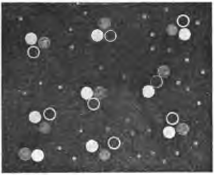


FIG. 34. ADSORPTION COMPOUND OF THREE CONSTITUENTS, as seen by ultra-microscopic observation of a mixture of fustic (white in the figure), Congo-blue (grey), and albumin (black, outlined by white).

The fustic particles actually were greenish in colour, those of the Congo-blue were red, and albumin yellow.

(After Raehlmann.)

Let us take, as the next case for consideration, a solid in mass immersed in a solution of some substance which lowers surface tension, and is, therefore, deposited on the surface of the solid. Further, let us suppose that this adsorbed substance is capable of entering into true chemical combination with the solid. It is clear that this reaction can only proceed at the surface, and it will depend upon the solubility of the products of the reaction whether the whole of the solid finally enters into combination, or whether there is merely a layer of the products on its surface. In any case, it is plain that the reaction will not obey the law of mass action in its usual form, since the rate of the reaction will depend, not on the mass of the solid, but on its surface.

Now, imagine the solid to be split up into smaller and smaller particles until they become molecules. At this point the ordinary law of mass action will be obeyed, since surface has no longer any existence.

This example shows the justification of the view taken by B. Moore (1909, p. 520), that there is no hard and fast line to be drawn between what he calls "molecular" compounds, which are the same as those called by others adsorption compounds, and true chemical compounds. In the same way, as we shall see in the next chapter, there are all stages of transition between colloids and crystalloids. This fact, however, does not alter the necessity of taking into consideration the surface energy of colloids and matter in mass. It appears that Moore desires to explain the phenomena of adsorption by chemical forces of an obscure and indefinite kind (see p. 534 of the article referred to), whereas it is known that there is present, and active, surface energy of various well-known forms, capable of satisfactorily explaining the characteristics of these phenomena without any further assumptions.

It seems to me that the well-known principle of logic called "William of Occam's Razor" may legitimately be applied to such a case as the one before us; "entia non sunt multiplicanda praeter necessitatem." Sir William Hamilton (1853, pp. 628-631) gives a more complete form in his "Law of Parsimony": thus "Neither more, nor more onerous, causes are to be assumed than are necessary to account for the phenomenon."

As physiologists, we must take the chemical or physical explanation, according to which leads further, when both are available. Some chemists appear to resent any explanation of a phenomenon apart from a chemical one. As has been pointed out above, the ultimate source of animal energy is almost entirely chemical, but, in the transportation and utilisation of this energy, physical factors intervene, and these factors cannot be neglected without serious error. Indeed, the same thing may be said of many non-vital processes, such as those of the galvanic cell or those taking place in surface films.

That there is, as Moore points out, a kind of stoichiometric relation between the constituents of adsorption compounds is not to be wondered at, if we remember the fact of adsorption saturation, that is, when the whole of the adsorbing surface is covered with the adsorbed substance. This relationship is between the extent of surface and the amount of compound formed and is not stoichiometric in the proper sense of the word. The amount adsorbed depends, not on the mass of the adsorbent, but on its state of subdivision, or its shape.

The constituents of living cells consist largely of substances in the colloidal state, so that it is not surprising to find that adsorption compounds are frequently to be met with amongst those extracted from these cells. Specially interesting are those in which lecithin is one of the components. When yolk of egg is extracted with ether, a compound of lecithin with vitellin goes into solution, although vitellin itself is insoluble in ether. Jecorin, again, a complex of glucose with lecithin and albumin, also appears to be an adsorption compound. It has been prepared by A. Mayer and Terroine (1907) by mixing solutions of acid albumin, lecithin and glucose all in dilute alcohol. The mixture is evaporated to dryness, extracted with ether, and precipitated from solution by absolute alcohol, just in the same way as Drechsel's original preparation from the liver. The other properties of this artificial jecorin are exactly those of the natural one. The fact that shows it to be an adsorption compound is that its composition varies with the relative proportion of the constituents of the mixture from which it is made. It has been claimed that jecorin can, by repeated precipitation and redissolving, be obtained of constant composition. It must be remembered, however, that this fact does not exclude adsorption. For one thing, if the whole of the constituents are precipitated by absolute alcohol, it is obvious that the precipitate will always have the same composition. Suppose further that we take electro-negative paper and allow it to adsorb night-blue, which is electro-positive. We find that, even from a moderately concentrated solution of the dye, practically the whole is taken up; so little is left that it would escape detection by analysis. Suppose that we dissolve this stained paper and re-precipitate it; in the second precipitation, practically the whole of the dye would go down again with the precipitate.

Another instructive case is the artificial laccase (an oxidising enzyme) prepared by Dony-Hénault (1908) by alcoholic precipitation of a solution containing gum arabic, manganese formate, and sodium bicarbonate. This precipitate can be redissolved in water and reprecipitated by alcohol. It is undoubtedly an adsorption compound of gum with colloidal manganese hydroxide. When the

gum, which acts as a protective colloid, ensuring fine subdivision of the manganese, in the way to be described in the next chapter, is thrown down by alcohol, it carries with it, in a state of adsorption, the manganese.

The inorganic salts, usually associated with proteins, are probably adsorbed. The law expressing the way in which they are removed by water shows that they are not merely admixed, while the fact that they *are* so removed shows that chemical combination is not in question (see my investigation of gelatine, Bayliss, 1906, pp. 179-185).

Several other compounds in which adsorption plays a part will be discussed in later pages.

It appears to be held by some observers that many of these adsorption compounds, especially those in which lecithin occurs, are more of the nature of *solid solutions*. The ratio in which their constituents stand to their concentration in the reacting mixture points rather to surface condensation, although solid solution cannot be entirely excluded. Loewe (1912, pp. 216-218) finds that the substances known as "lipoids," of which lecithin is an example, take up dyes, hypnotics, and tetanus toxin in a way which is not compatible with the solid solution views but with an adsorption process. The exponents of the equations, expressing the relation of the amount taken up to the concentration of the solutions, are not of such values as to admit of the interpretation of distribution between phases in unequal proportion. Moreover, when nicotine or methylene blue in solution is allowed to remain for a long time in contact with lipoid matter, no diffusion is found to take place into the interior of the lipoid. It appears, therefore, that the action is a surface one.

ADSORPTION AS CONTROLLING CHEMICAL ACTION

That adsorption does not preclude subsequent true chemical combination is obvious. So far is this the case that, in many cases, chemical change seems to necessitate preliminary adsorption. One at least of the constituents of an adsorption compound possesses, of course, a surface, either the visible one of such materials as paper, cell granules, and various fabrics or tissues, or the ultramicroscopic surfaces of colloidal particles. Substances in such states of aggregation are naturally inert, as far as chemical activity is concerned, so that when the chemical reaction is between the components of the phase possessing the surface and the adsorbed substance, it is to be expected that it will proceed very slowly.

O. C. M. Davis (1907) finds that charcoal takes up iodine with great rapidity up to a certain point of apparent equilibrium, but that, if the components are allowed to remain together for a longer time, a very slow further disappearance of iodine goes on. The first part of the process differs, as would be expected, according to the particular kind of charcoal used, since the surfaces would vary. The second process is the same for various kinds of charcoal and is interpreted by Davis himself as being a passage of iodine into the mass of the solid; a solid solution, in fact. It is suggested by Freundlich (1909, p. 173) that chemical combination is more probable, since iodine is a very reactive substance. This suggestion explains why the second part of the process is irreversible and does not vary with the kind of charcoal used.

It is probable that the fixing of dyes on tissues by heat is due to chemical combination. When Congo-red is taken up by filter paper in the absence of electrolytes, it is readily washed out again. But if raised to 100° it becomes fixed. The same process goes on slowly at ordinary temperatures.

There are two classes of reactions in which the rate of chemical combination is controlled by adsorption. The first is when the two reacting substances are condensed on the surface of a third and combine together there, leaving the adsorbing surface in the end unaltered. This process is one of those that we shall learn later to call "catalytic." Examples of such reactions are:—

(1) The production of sulphuric acid under the influence of platinum, in which it has been shown by Bodenstein and Fink (1907) that the rate of the reaction is governed by the adsorption of SO_2 on the surface of the platinum.

(2) The effect of platinum on the reduction of titanic sulphate by hydrogen (Denham, 1910).

(3) The decomposition of ozone by heat takes place on the walls of the containing vessel, or other surface present (Perman and Greaves, 1908).

The second class of cases is typified by that of a colloidal hydroxide and colloidal acid, as described above. The reacting substances are first brought together by mutual adsorption, and chemical reaction then follows between the whole of the constituents of the system. A very similar case is described by van Bemmelen (1910, p. 486). If barium hydroxide solution be added to colloidal silica, a white precipitate falls, which is found to contain both barium hydroxide and silica, but not in chemical combination. On standing, barium silicate is slowly formed and crystallises. Another case is the action of tannin on leather. According to Freundlich (1909, p. 532), the amount of tannin taken up is conditioned by an adsorption process, which is then followed by true chemical reaction, which takes place slowly and results in the formation of insoluble bodies.

The fact to be insisted upon in these cases where chemical reaction follows adsorption is that the velocity of reaction, as affected by various conditions, does not follow the law of mass action in its usual form. The active mass here is the amount adsorbed on the surface, so that the reaction as a whole will be observed to follow the parabolic law of adsorption. The systems of chief interest to the physiologist are those of which colloids form part. Although these are heterogeneous systems, the internal or dispersed phase is so minutely divided and evenly distributed that, in comparison with the cases investigated by Nernst, the rate of diffusion does not appear to play so important a part. We shall have to return to this aspect of the question when treating of enzymes.

This is perhaps the most appropriate place to refer to some cases of biological interest which illustrate the manner in which adsorption intervenes in a variety of processes.

1. The power of the soil in holding back soluble salts, so that valuable foods are not washed away by the rain. Shown by the experiment with sand and permanganate solution given by J. J. Thomson (1888, p. 192).

2. Dr Harriette Chick has shown (1906, p. 247) that the complex organic substances, which are detrimental to the nitrifying organisms in the filter process of sewage treatment, are kept back by adsorption in the upper layers of the filter bed.

3. The action of certain poisons on micro-organisms has been found to be proportional to the amount deposited on their surfaces (H. Morawitz, 1909, pp. 317-322).

4. Craw (1905) has shown that the combination between toxin and antitoxin follows more closely as to its laws the phenomena of adsorption than those of chemical combination. Perhaps the most striking fact in this connection is the explanation given of the puzzling phenomenon of Danysz (1902), who found that when a given quantity of diphtheria toxin was added in fractions to antitoxin, more toxin was neutralised than when the same quantity was added at one time.

To neutralise ricin, the toxic substance from the castor bean, it was found that less antiricin was necessary if added to a definite amount in successive quantities than if added all at once. And, if ricin be added in separate doses to a definite amount of antiricin, the same amount of ricin requires more antiricin to neutralise it than if the whole is added at one time.

This phenomenon also takes place when paper adsorbs Congo-red (Bayliss, 1906, p. 222). The explanation is that the same amount of adsorbent will take up relatively more from a dilute solution than from a more concentrated one.

5. In the taking up of bacilli by leucocytes under the influence of a sensitising fluid (so-called "opsonin"), it was found by Ledingham (1912, p. 359) that the two processes involved both followed the course of an adsorption process. These two parts of the phenomenon are (1) the taking up of "opsonin" by the bacilli, and (2) the ingestion by the leucocytes of the micro-organisms thus "sensitised."

6. When the toxin of tetanus is introduced into a nerve trunk of a warm-blooded animal, it is carried up to the central nervous system and produces convulsions in due course. If the same experiment be performed on a frog at 8° C. it was found by Morgenroth (1900) that although taken up by the nervous system, no convulsions were produced until the animal was warmed to a temperature of about 20° C. This is evidently a similar case to that of the Congo-red acid and thorium hydroxide described above. The toxin, although adsorbed, exerts no action until chemical reaction of some kind takes place on warming.

7. The rate of action of enzymes is controlled by adsorption, but full discussion will be more conveniently deferred until later.

8. When the protoplasmic contents of a ciliate infusorian or the root hair of a plant are pressed out into water, a membrane is at once formed on the free surface of the protoplasm. This fact has been described by Kühne (1864, p. 39) and by Pfeffer (1897, i. pp. 92, 93). The nature of this membrane will be discussed in Chapter V., and it will suffice to call attention to it here as being undoubtedly due to surface concentration of cell-constituents which lower surface energy.

9. The blue substance formed by the action of iodine on starch has long been familiar, but its nature as an adsorption compound has only recently (1912) been made clear by the work of Barger and Field (1912). They also show that similar blue compounds are formed by substances of very varied chemical nature, such as saponarin, cholalic acid, and lanthanum acetate.

10. That powerful action on cell processes can be exerted by substances which do not penetrate beyond the surface of the cell is shown by a very interesting experiment of Warburg (1910, pp. 310, 311, 313). The oxygen consumption of the fertilised eggs of a sea-urchin in an artificial sea-water is doubled by the addition of 10 c.c. of decinormal sodium hydroxide to 1 litre of the sea-water, the development being, at the same time, stopped. If the cells are stained previously with neutral red, which does not affect their development, no change of colour takes place on addition of sodium hydroxide; whereas with ammonia, to which the cell membrane is permeable, the cells become yellow in less than one minute. Athough uninjured by the concentration of ammonia used, the oxygen consumption is only increased by 10 per cent. instead of the 100 per cent. when the $H^{\cdot}$ ion concentration is changed only at the surface.

THE CONDITION OF ADSORBED MATERIAL

There is one point that it is of some importance to understand clearly. When an electrolyte, say acetic acid, is adsorbed by charcoal, it is *fixed* for the time on the surface. By this statement it is not meant to imply that the same identical molecules remain in the same place, but that a certain proportion of the acid is taken out of solution and cannot take part in such properties as the electrical conductivity or the osmotic pressure of the system. A mixture of acetic acid and charcoal has the electrical conductivity of the liquid phase alone. Similarly, when the particles of the adsorbent are too large to give an osmotic pressure (see Chapter V.), the osmotic pressure of the system is due only to the solution. The adsorption compound of charcoal plus acetic acid, or other adsorbed electrolyte, has no higher osmotic pressure nor conductivity than the charcoal itself. Some observers are inclined to attribute, incorrectly, the osmotic pressure undoubtedly shown by certain colloidal solutions to adsorbed electrolytes or crystalloids.

In the state of adsorption, salts, not being electrolytically dissociated, give none of their characteristic reactions. Iron, for example, is in what is sometimes called a "masked" condition.

Ruer (1905) found that when chlorides are adsorbed by colloidal zirconium hydroxide, no reaction with silver nitrate is given. The presence of chlorine in colloidal ferric hydroxide can only be detected by transforming the colloidal solution into a true solution by means of nitric acid, that is, by abolition of the adsorbing surface.

On the other hand, it must not be forgotten that adsorbed substances are only fixed as long as the solution with which they are in equilibrium remains of the same concentration, which may, however, be very low. Nevertheless, by repeated washing, practically the whole of the adsorbed matter may be removed, although an infinite number of changes of water is theoretically necessary. If charcoal which has adsorbed sugar be placed inside an osmometer, whose membrane is permeable to water and sugar, but not to charcoal, sugar will pass out to water on the outside, and by repeated changes of this water the sugar can be almost entirely removed from the charcoal inside. Substances merely adsorbed cannot be prevented from escape to water; in order that they shall not do so, they must

be in a state of non-dissociable chemical combination with the substance to which the membrane is impermeable. Many substances, such as many dyes, must be solid in the adsorbed state (see Willows and Hatschek, 1915, p. 47).

ADSORPTION FROM MIXTURES

When adsorption takes place from a mixture, all solutes are taken up and in definite relative proportions. Not only so, but, as Williams (1913), working in the laboratory of Arrhenius, has pointed out, the solvent itself is condensed on the surface. This leads to a complex state of affairs, which has not yet been worked out completely. As we shall see later, the facts have an important bearing on the state of equilibrium arrived at under the action of enzymes.

If a surface which has adsorbed a particular substance be exposed to a solution of another one which has a greater power of lowering surface energy than the first, there is a more or less complete displacement of the less powerful one by the other.

This is shown in an interesting way in the experiments of Schmidt-Nielsen (1910, p. 342). When rennet is shaken up in solution, it is more or less inactivated by adsorption on the surface of the froth produced. This inactivation is completely absent if a little saponin be added, although the foam is even greater than before. Saponin, in fact, lowers surface energy more than does rennet, hence it obtains possession of the surface. The same fact is seen in the driving out of rennet from its adsorption by charcoal in the experiments of Jahnson-Blohm (1912). Charcoal added to rennet prevents its action on milk (acting as an anti-enzyme), but, if saponin be added to such an inactive mixture, it becomes active owing to the driving off by the saponin of the rennet from its "combination with the antibody."

This fact, that one substance can displace another from adsorption, is of importance with respect to the turning out of oxygen from oxyhæmoglobin by exposure to carbon monoxide (see Chapter XXI.).

DYEING AND STAINING

Many facts have been mentioned in the preceding pages which indicate the important part that surface action, or adsorption, must play in these processes, as well as the probability that chemical reaction may, in many cases, follow it, although adsorption is the controlling factor.

Weber (1894) finds that the amount of dye taken up by cellulose is in proportion to the extent of surface presented by the latter. Precipitated cellulose takes up more than does an equal weight of compressed paper. Dinitrocellulose, freshly precipitated, adsorbs in about the same degree as ordinary cellulose; but in the form of a coherent film little or none is taken up.

In discussions on the subject of staining, the use of the names "*basic*" and "*acidic*" is liable to lead to some misconception. With one or two exceptions, all dyes are neutral salts; the distinction is that the so-called "basic" dyes are salts of an organic coloured base with an inorganic acid, usually hydrochloric, although sometimes salts with acetic acid are met with. The "acid" dyes, on the other hand, are salts of a coloured organic acid with an inorganic base, usually sodium.

Bearing this fact in mind, it is clear that, if a "basic" dye stains a particular cell constituent, it does not directly follow that this constituent is an acid. If such, it must be a stronger acid than that combined with the colour base of the dye, usually hydrochloric. Double decomposition may occur, of course, if the cell constituent in question is a salt. This will be more complete the less soluble the compound between dye and tissue is. Similar statements apply, *mutatis mutandis*, to "acidic" dyes. Since most of the staining bodies in cells are colloids and with negative charges, it is easy to understand why electro-positive dyes, such as many of the "basic" ones are, should be adsorbed. It is also suggestive that hæmoglobin, one of the few electro-positive colloids of the organism (Iscovesco, 1906), takes up "acid" dyes, such as eosin and acid fuchsin. Moreover, when the dye salts are electrolytically dissociated, as in most cases, the positive ion is the coloured one in the "basic" dyes, and will be taken up by negative surfaces, while the negative ion of the "acid" dyes will be taken up by the positive surfaces. The "basic" dyes are frequently hydrolytically dissociated, with formation of electro-positive free bases in the colloidal state.

There are some more facts of interest, tending to show the great importance of the electrical charge of the surface. Gee and Harrison (see William Harrison, 1911, p. 6 of reprint) found that the maximum negative charge of cotton, wool, and silk was at a temperature of 40° C. Brown (1901, p. 92) had previously shown that the maximum adsorption of "basic" (electro-positive) dyes by wool took place at the same temperature.

W. Harrison (1911, p. 26) also showed that cotton treated in various ways, nitrated, mercerised, and so on, had a contact potential difference against dilute sodium chloride which differed considerably in amount according to the treatment, although the charge was always negative. The amount of "acid" (=electro-negative) dye adsorbed was parallel with the *decrease* in the charge.

That the deposition of an electro-positive dye on a negative surface results in a lowering of the charge on this surface is shown by an experiment of Larguier des Bancels (1909). The charge on wool, as measured by the number of drops of water transferred from one electrode to the other, in an apparatus similar to that of Perrin (1904, p. 616), in a given time was represented by 77. After staining with methylene blue, the number was reduced to 18.

The very marked effect of electrolytes in altering the charge on surfaces has been frequently referred to, as also the fact that electro-negative substances are scarcely adsorbed at all by electro-negative surfaces. In order that this adsorption may take place, the surface must be discharged, or the amount of its charge lessened, by the action of an ion of opposite sign. That very small quantities of an appropriate ion suffice is well shown by an experiment of Elissafoff (1912, p. 404), whose work will be referred to in more detail in the chapter on "The Colloidal State." 0·2 mgm. of thorium nitrate per litre lowered the charge on the surface of a quartz capillary by 50 per cent.

The absence of staining by "acid" dyes in the absence of electrolytes explains why fresh teased nerve fibres of the frog only stain with Congo-red at their cut ends, where, according to Macdonald (1905, p. 329), electrolytes are set free. Emil Mayr (1906, p. 560) finds that the affinity of Nissl bodies in nerve cells for "basic" dyes is reduced by previous treatment with neutral salts. This fact also is in agreement with the doctrine of electrical adsorption. The Nissl bodies have, in all probability, a negative charge; this charge would be diminished by cations, and hence the attraction for positive substances, like the "basic" dyes, would be also diminished.

Disregard of this action of electrolytes has led to certain erroneous statements with regard to dyes. It is to be remembered that commercial specimens almost invariably contain a large percentage of salts, frequently as much as 20 to 30 per cent. of sodium chloride or sulphate, arising from the mode of preparation. When it is said that Congo-red is a "direct" dye for cotton, the statement only applies to the commercial dye, with its content of salts. When adsorption, moreover, takes place under the influence of electrolytes, it is, as a rule, "faster," that is, not so easily removed by the action of water, than when it takes place in their absence. This applies more especially to the electro-negative dyes.

Certain facts described as "anomalous adsorption" (Biltz, 1910) will also be found to be explained by the presence of electrolytes (Bayliss, 1911, 3).

For many purposes it is necessary to have *pure dyes*. The following method, due to Harrison (1911, p. 17), may be recommended. It depends on the displacement of the non-volatile salts, present as impurity, by a volatile one. The dye in concentrated solution is precipitated by saturation with ammonium carbonate ("salted out"), redissolved in water, and again salted out. After washing with a saturated solution of ammonium carbonate, the precipitate is dried at 110° C., when all the ammonium carbonate is driven off.

A curious fact was noted by Freundlich and Losev (1907, pp. 311, 312): When an "acid" dye is adsorbed, the whole of the molecule is taken up. When a "basic" dye is adsorbed, the positive coloured ion only is taken up, leaving the acid. Satisfactory explanation of these facts is not at present at hand (see Freundlich and Neumann, 1909). There are, however, two facts to be remembered in this connection. The "acid" dyes are, as a rule, sodium salts of strong (sulphonic) acids and are very little, if at all, hydrolysed in solution, but electrolytically dissociated to a considerable degree. The anion, containing a large number of atoms, seems to behave as a colloid and has, of course, a negative charge. The "basic" dyes, on the other hand, are salts of a rather feeble organic colour base with a strong acid and are hydrolysed in solution. The free base is insoluble, in the ordinary sense, but forms a colloidal solution, the particles having a positive charge. The behaviour of the two classes may perhaps depend in some way on this difference in mode of dissociation.

SUMMARY

The surface of contact between a liquid and another phase—solid, immiscible liquid, or gas—has properties differing from those of the main body of either phase.

In the first place, the surface film behaves as if stretched, so that it is the seat of a special kind of energy.

This surface tension has its origin in the forces of attraction between the molecules of the liquid, the forces which give rise to the internal pressure of Laplace.

The amount of this surface energy varies with the chemical nature of the liquid.

All solutes, with the exception of certain inorganic salts, lower the surface tension at the interface between liquid and air; these particular salts do so at the interface between liquids.

The interface between phases is also nearly always the seat of electrical forces, the origin of which is usually from electrolytic dissociation in one or other of the phases. But the possibility of phenomena akin to those of frictional electricity cannot as yet be definitely excluded.

Solubility is also changed in the surface film.

Since any process that diminishes free energy tends to occur, a solute will be found in higher concentration in the surface film than in the body of the liquid if it has the power of reducing surface energy. By this means, a greater fall in surface energy is ensured. (Principle of Willard Gibbs.)

This surface condensation is known as "adsorption" and plays an important part in physiological phenomena. The surface energy spoken of in the previous statement of the Gibbs principle may be of many kinds, mechanical, electrical, chemical, etc.

In certain cases, surface concentration leads to the formation of a more or less rigid film, as, for example, with saponin or proteins (Ramsden).

When a solute has an electrical charge, either as an ion or as a colloidal particle, and the surface in contact with the solution has also a charge, the degree of adsorption depends on the relative sign of the two charges; no decrease of free energy would be produced by adsorption of a negatively charged substance on a similarly charged surface, but the reverse. On the other hand, adsorption of an oppositely charged substance leads, by neutralisation of the charge, to decrease of free energy.

If the surface has no charge, while the adsorption of an electrically charged ion would lead to diminution of mechanical surface energy, such adsorption will take place and cause the appearance of an electrical charge on the surface.

Adsorption of a similarly charged ion or colloid can be increased by reversing the sign of the charge on the surface by allowing it previously to adsorb ions of sign opposite to itself. If the surface and the solute have already opposite signs, it is clear that previous adsorption by the surface of an opposite charge will decrease subsequent adsorption of the particular solute.

These phenomena of electrical adsorption play a considerable part in the processes of dyeing and of histological staining.

Chemical reactions which lower chemical potential are also favoured at a surface. The rate of such reactions is not controlled by the total mass of the reagents, as in true solution, but by the extent of active surface. The law of mass action, in its simple form, does not apply quantitatively, since the surface of one or both of the reagents has to be taken into account.

There is some evidence that the chemical configuration of the surface may play a part in adsorption and lead to the appearance of "specific" action. But the question needs further investigation.

The rate at which adsorption takes place, when the components are already approximated, appears to be very rapid, although not instantaneous. On the other hand, when the substance to be adsorbed has to diffuse from distant parts of the system, the rate will be controlled by diffusion and therefore accelerated by rise of temperature.

The total amount adsorbed in equilibrium is *less* the higher the temperature. The process, by the "principle of mobile equilibrium," is, therefore, associated with the production of heat.

The mathematical form of the expression relating concentration with amount adsorbed is a characteristic one and belongs to the parabolic family.

Adsorption cannot be completely or satisfactorily explained by chemical combination, nor by partition between phases, in accordance with relative solubility, since impossible assumptions have to be made as regards molecular association, etc.

A class of compounds exists in which, as shown by various facts, the constituents are not chemically combined. This is shown especially by the dependence of their composition on the relative concentration of the substances from which they are produced. This class of compounds is satisfactorily explained by adsorption.

In some of these adsorption-compounds the constituents can be shown to be present, side by side, but uncombined.

Since the rate of chemical action depends on the concentration of the reagents it is plain that when a substance is capable of reacting with a second one, which is present as a separate phase, particles or drops, for example, the rate of reaction will depend on the amount of the one adsorbed on the surface of the second. Similarly, if two substances, capable of reacting with each other, are both adsorbed on the surface of a third, with which they do not combine, their rate of reaction with each other will be accelerated by the increased concentration, or molecular approximation, due to adsorption.

A number of cases are given where adsorption plays a controlling part in phenomena of physiological interest.

Salts when adsorbed are not electrolytically dissociated and do not therefore give their characteristic reactions, neither can they be osmotically active.

A substance which lowers surface energy more than a second one does will drive off this latter from adsorption on a surface, at the same time taking its place thereon.

From a mixture, all constituents, including the solvent, are condensed on a surface and in definite proportions.

Adsorption plays a large part in the phenomena of dyeing and staining; most, if not all, of the facts can be explained on this basis; although, in all probability, chemical reaction sometimes follows adsorption, the rate of this reaction being controlled by the amount adsorbed.

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Boys (1912).
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Adsorption.
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Wolfgang Ostwald (1909, pp. 422, 433).
Perrin (1904, 1905).
Freundlich (1909, pp. 184-265).
Bayliss (1906, 1).

Dyeing and Staining.
A. Fischer (1899, pp. 73-201).

Adsorption preliminary to Chemical Action.
Bayliss (1911, 1).

CHAPTER IV

THE COLLOIDAL STATE

IF we take a piece of metallic gold, immerse it in water, and divide it up into smaller and smaller parts, it is obvious that in the end, supposing that our powers of manipulation were adequate, we should arrive at the molecular condition. But, before this state is reached, we should have passed through a state in which the particles were so fine as to be invisible, as such, by ordinary means of illumination; and they would remain in permanent suspension, so as to simulate very closely a true solution, in which the substance dissolved is in the molecular, or even ionic state. In the course of this process of division, the larger fragments of gold of the early stages sink at once, after being stirred up, but as smaller and smaller particles are formed, the time taken to fall becomes longer and longer, until, when less than a certain size, they do not appear to sink at all. They are now in what is called the "*colloidal state.*" Their dimensions at this stage are enormously greater than those of molecules of gold, but it is clear that we can draw no definite lines of demarcation between the visible solid lump, from which we started, the colloidal state and the final molecular state.

We cannot, of course, actually perform the operation in the manner described. In an indirect way, however, it was done by Faraday (1858, p. 159), who found that, by acting on solutions of gold salts by reducing agents, beautiful red or purple solutions were obtained. He also showed that these solutions, although permanent, were, in reality, suspensions of minute particles of metallic gold (p. 160 of the above paper). It is interesting to note that one of Faraday's gold preparations is still preserved in the Royal Institution.

Since these gold solutions have served as the foundation for much subsequent work, the method of preparing them is worth description. The ruby-red solution is made thus, in the words of Faraday himself (1858, p. 159): "If a pint or two of the weak solution of gold before described" (*i.e.*, about 2 grains of gold chloride in two or three pints of water) "be put into a *very clean* glass bottle, a drop of the solution of phosphorus in sulphide of carbon added, and the whole well shaken together, it immediately changes in appearance, becomes red, and being left for six to twelve hours, forms the ruby fluid required; too much sulphide and phosphorus should not be added, for the reduced gold then tends to clot about the portions which sink to the bottom." Zsigmondy (1905, pp. 97-101) finds that the method is improved by the addition of potassium carbonate, in order to neutralise the free acid produced in the reaction; he also gives other useful hints, pointing out the importance of pure water and Jena glass vessels; the absence of colloidal matter from the water used appears to be especially necessary if uniform results are to be obtained. The necessity of cleanliness was well known to Faraday himself, although at that time the properties of colloids were unknown.

A beautiful deep blue solution of gold can be made by reduction with hydrazine hydrate (Gutbier, quoted by Svedberg, 1909, i. p. 10). Gold chloride 0·1 per cent. is neutralised by sodium carbonate and very dilute hydrazine hydrate (one part in 4,000 of water) added drop by drop, carefully avoiding excess.

How do we know that we have to do with suspended solid particles in these preparations? They are quite transparent to light of ordinary intensity, although this does not apply to all colloidal solutions; where the particles are larger the solutions are turbid, and their appearance suggests their nature. Even the most transparent gold preparations, however, were found by Faraday to show turbidity in the track of a powerful beam of light. This observation forms the foundation of the ultra-microscope, to be described later. It is frequently called the "Tyndall-phenomenon," but its discovery was really made by Faraday (1858, p. 160). Tyndall pointed out that the light reflected, or rather diffracted, from the path

of the beam is polarised, a fact which proves that the particles are of the same order of dimensions as the mean wave length of the light used.

A further proof that we have to do with suspended particles is given by Friedenthal (1913). By powerful centrifugal force, he has separated several colloids from solution, caseinogen from milk, for example. Iodised starch, mixed with non-iodised, could be separated from the latter, owing to its greater weight.

The colloidal state, then, is of the nature of a heterogeneous system, or a system of more than one separate phase. The point of importance to be remembered is that the phases of which the system consists are separated from one another by surfaces, interfaces, of contact. The colloidal state differs from a coarsely heterogeneous system, such as a mass of gold immersed in water, in that it is, to ordinary observation, homogeneous, and only shows its micro-heterogeneous character by special methods of investigation. On the other hand, it is distinguished from true solutions of small molecules or ions by the fact of the possession of surfaces of contact, with all the phenomena implied by this. These properties will naturally be especially marked on account of the great surface area due to the minute state of subdivision.

It is convenient to have names for the two phases of which a colloidal system usually consists. If we refer back to Fig. 15 (page 14), we see the appropriateness of Hardy's names (1900, 2, p. 256), of "external" and "internal" phases. Other workers call Hardy's internal phase the "dispersed phase," and the external phase the "continuous" one (Wo. Ostwald, 1907, p. 256). The names will be used here indifferently.

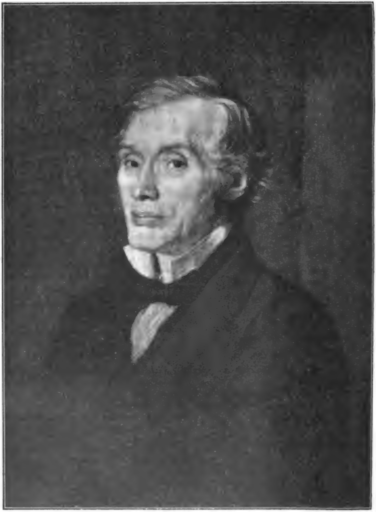
One essential condition for the production of a colloidal solution of a substance is that it should be practically insoluble in the external phase, or "dispersing medium," to use another expression of frequent usage. This statement, however, needs some qualification, as we shall see later. It is especially insisted on by von Weimarn (1911, p. 6) that, given appropriate conditions, all substances can be brought into the colloidal state.

It may be mentioned, as an illustration, that resinous substances like gamboge or mastic form true solutions in alcohol, but when such solutions are poured into water, a colloidal solution is produced. The same investigator gives strong evidence to show that, conversely, all substances can, by appropriate manipulation, especially very slow deposition, be obtained in the crystalline form (1912); although the crystals of such liquid or semi-liquid substances as proteins are apt to be very minute and distorted in shape, rounded at the edges, by the action of surface tension.

Most of our knowledge of the fundamental properties of the colloidal state is due to Thomas Graham, whose portrait will be seen in Fig. 35.

Graham started from a different point of view from that of Faraday. He noticed that certain substances are extremely slow to diffuse, and devoid of the power to crystallise (1861, p. 183). They are also unable to pass through a membrane of similar nature to themselves, such as sized paper or parchment paper (unsized paper treated with sulphuric acid). Amongst these substances are hydrated silicic acid, starch, albumin, gelatine, etc. He says (1861, p. 183): "As gelatine (κόλλη = glue) appears to be its type, it is proposed to designate substances of the class as *colloids*, and to speak of their peculiar form of aggregation as the *colloidal condition of matter*. Opposed to the colloidal is the crystalline condition. Substances affecting the latter form will be classed as *crystalloids*. The distinction is no doubt one of intimate molecular constitution." It will be noted that, although Graham speaks here of the "colloidal condition" of matter, he appears to regard the class of colloids as quite distinct from that of crystalloids. "They appear like different worlds of matter" (1861, p. 220). At the same time he is aware that the same substance, silica for example, may be obtained in either state, while on the page following that on which the above statement is found, he suggests that the colloid molecule may be "constituted by the grouping together of a number of smaller crystalloid molecules." Perhaps stress is intended to be laid rather on the word "appear." In any case, it is better to speak of the "colloidal state" and not of "colloids" as a class.

One important characteristic of this state, that of *instability*, was clearly recognised by Graham. After referring to the fact that colloidal solutions of



Thos: Graham

FIG. 35. THOMAS GRAHAM.

The signature is taken from the facsimile reproduction of the Charter Book of the Royal Society.

(From the portrait by G. F. Watts in the rooms of the Royal Society. By permission of the Council.)

silica sooner or later become gelatinous and finally crystallise, he says (1861, p. 184): "The colloidal is, in fact, a dynamical state of matter; the crystalloidal being the statical condition. The colloid possesses ENERGIA. It may be looked upon as the probable primary source of the force appearing in the phenomena of vitality. To the gradual manner in which colloidal changes take place (for they always demand time as an element), may the characteristic protraction of chemico-organic changes also be referred." This "energia" we know now as "surface energy" of its various kinds.

The two phases of which a colloidal solution consists may obviously be of many various kinds. The table below will illustrate this:—

Internal or Dispersed Phase.	External or Continuous Phase.	Example.
1. Gas - - - -	Liquid - - - - -	Foam.
2. Liquid - - - -	Gas - - - - - -	Fog.
3. ,, - - - -	Another immiscible liquid	Emulsion or emulsoid; milk.
4. ,, - - - -	Solid - - - - -	Jelly, as gelatine in some forms.
5. Solid - - - -	Gas - - - - - -	Tobacco smoke.
6. ,, - - - -	Liquid - - - - -	Ordinary colloidal solution, such as those of gold, arsenious sulphide, etc.
7. ,, - - - -	Another solid - - -	Ruby glass.

The most important systems for the physiologist are those consisting of solids and liquids, Nos. 3, 4, and 6. The nature of the dispersed phase as solid or liquid has been adopted as a basis of classification by Wo. Ostwald (1907, p. 334). This system is in many ways a useful one, although it does not direct attention to what is perhaps the most important distinction between different classes, that is, the affinity of the dispersed phase for water. When the internal phase, although liquid, is in extremely minute droplets, its mechanical properties closely resemble those of a solid—the great pressure due to the internal component of the surface tension confers rigidity on them. The characteristic which carries with it most of the other differences in the general behaviour of a colloidal system is the affinity of the internal phase for water, or other solvent, constituting the external phase. It will be clear that the more water the internal phase contains, and it may contain as much as 90 per cent., the less will be the difference between the properties of the two components of the interface of contact between it and the external phase, and, consequently, the less will be the surface energy.

Hardy (1900, 1, p. 236) calls attention to the fact, also pointed out by Quincke (1902, p. 1012), that, as a rule, the material of which the internal phase is composed is not absolutely insoluble in the external phase, so that the two phases will be (1) a solid containing a certain amount of the solvent, and (2) a very dilute true solution of the solid. The substance of which the solid phase is composed will become more soluble, as a rule, as the temperature is raised. This fact is sometimes of use as a means of indicating whether the external phase of a colloidal solution does actually consist of a dilute true solution of the substance in suspension. The most convenient way of detecting this is by measuring the electrical conductivity. However long a colloidal solution has been dialysed (a means of purification to be described later, and depending on the impermeability of certain membranes for colloids), it is almost impossible to remove all traces of foreign electrolytes. Now, as the temperature is raised, these foreign bodies will not increase in number; since the impurity is in extremely low concentration, it may be regarded as being completely dissociated electrolytically. The increase in conductivity, so far as it depends on this impurity, will be due only to the increased rate of movement of the ions already present, dependent on the diminished viscosity of the solvent. The temperature coefficient of this is known, and lies between 2 and 2·4 per cent. of the conductivity at 18° per degree rise of temperature. Suppose we take a solution, saturated at 18°, of an electrolyte, for convenience a somewhat insoluble one, such as sulphanilic acid, and determine its conductivity at various temperatures, we find the temperature coefficient to be 2·6. But if excess of undissolved acid is present, more and more will go into solution as the temperature is raised, the actual number of ions is increased, and the temperature coefficient appears to be considerably higher, viz., 5·9. Applying this fact to the colloidal system, if the conductivity is due to foreign ions, the temperature coefficient will be only 2 to 2·4, and if it is found experimentally to be higher than this, evidence is afforded that more of the colloidal substance itself goes into true solution. The free acid of Congo-red is a

case in point. Here, as I find, the temperature coefficient is either 3·6 or 7·3, according to whether the measurements are made from higher to lower, or vice versa. The difference is, no doubt, due to hysteresis (see below). The measurements were made in a quartz vessel. The hypothesis can be tested in another way, not so satisfactory in practice. If a dilute solution of an electrolyte be further diluted, say to twice its volume, its conductivity will be halved, because no new ions will be produced. If the conductivity of a colloidal solution be due to traces of electrolyte impurity, on dilution its conductivity will be reduced in exactly the same proportion. Whereas, if due to slight true solubility of the colloid itself, it will remain unaltered; or at all events, less diminished than in ratio to the dilution. There is always excess of the solid phase present, so that the external phase is always a saturated solution. If the particles diminished in size, owing to further subdivision, greater dispersion, it is even possible that the conductivity might rise on dilution, owing to the greater solubility of fine particles. The experiments of Hulett (1901, p. 406) show the solubility of barium sulphate in particles of 1·8 μ to be 2·29 millimoles per litre, in particles of 0·1 μ to be 4·15 millimoles per litre. This fact is in agreement with the experience of chemists that large particles in precipitates grow at the expense of smaller ones; or from a mixture of crystals, deposited from a hot saturated solution when it cools, the smaller crystals gradually disappear while the larger ones increase in size. The fact is connected with the diminution of surface energy involved in the process.

Perrin (1905, p. 85) divides colloidal solutions into "hydrophile" and "hydrophobe," according to the affinity of the dispersed phase for the water; "*lyophile*" and "*lyophobe*" would be better, as Freundlich points out, since water may be replaced by other solvents. This classification is almost coterminous with that of Hardy (1900, 1 and 2) into reversible and irreversible colloids, according to whether, after evaporation to dryness, they go into solution again on mere addition of water or remain as a solid film. Typical instances of the hydrophile class are gelatine and gum, of the hydrophobe class, gold and arsenious sulphide. Intermediate forms are also known to exist, that is, systems which have some of the properties of each class. Such are the sulphur preparations of Sven Odén (1912, p. 712), which give reversible precipitates with salts, like the hydrophile class, but are precipitated by very small concentrations of bivalent ions, like the hydrophobe class. It must be admitted that the existence of these intermediate kinds of colloidal systems deprives all classifications as yet proposed of much of their theoretic value, although useful in practice.

The names "*sol*" and "*gel*" introduced by Graham (1864, p. 321, p. 620 of the Collected Edition, 1876) may be referred to here; a colloidal solution of silicic acid, at first liquid, becomes gelatinous in process of time. The two states are called "hydrosol" and "hydrogel" respectively, when the external phase is water. When this is alcohol, "alcosol," and so forth.

Some degree of confusion is apt to arise from the use of the words "homogeneous" and "heterogeneous" as applied to solutions. It is plain that no solution can be absolutely homogeneous; a molecule of water and one of sodium chloride cannot be in the same place at the same time. Indeed, von Calcar and Lobry de Bruyn (1904, p. 218) thought that they had succeeded in producing, by centrifugal force, changes of concentration in solutions of potassium iodide. It is also clear that, if we make as our criterion of heterogeneity the power we possess of separating the phases mechanically, as Bakhuis Roozeboom (1901, p. 9) does, colloidal solutions cannot be called heterogeneous. The really important point is, following the work of Willard Gibbs, whether the phenomena due to the possession of surfaces of contact, as shown by matter in mass, are also shown by the "particles" of the internal phase in colloidal solutions. About this there is no dispute; but, to avoid misunderstanding, it is perhaps advisable not to use the name "heterogeneous" in their case, and to speak of colloidal solutions as "micro-heterogeneous," one or more of the phases being minutely subdivided.

Where then can we say that "molar" properties cease and "molecular" properties begin? The question remains as yet unanswered, but it seems clear that a gradual transition must exist, and possibly some of the disputes as to the relation between the chemical and physical properties of certain colloidal systems may be due to an exclusive consideration of a part only of the phenomena shown by these intermediate states.

Whatever phenomena are manifest at interfaces between phases will obviously be greater as these interfaces increase in area. It is of interest, therefore, to calculate the amount by which the surface of a given mass increases when subdivided to colloidal dimensions. The particles of gold in some of the preparations of Siedentopf and Zsigmondy (1906) were found, by a method to be described

later, to have a radius of about one-millionth of a centimetre. A sphere of gold of one-tenth of a centimetre radius has a surface of 0·126 sq. cm., while the surface of the same mass, if subdivided to the above colloidal dimensions, would have a surface of about 100 sq. m., or be multiplied by ten millions.

It will occur to the reader that we are very near molecular dimensions in the case of these finest particles. In fact, Siedentopf and Zsigmondy obtained gold hydrosols with particles of less than 6 $\mu\mu$ in diameter (μ is 0·001 mm., and $\mu\mu$ is one-thousandth of this, *i.e.*, one-millionth of a millimetre), while starch is stated by Lobry de Bruyn and Wolff (1904) to have a molecular diameter of 5 $\mu\mu$, and even carbon dioxide has a value of 0·29 $\mu\mu$ (Nernst, 1911, p. 434).

In practice it is found that Graham's criterion of not passing through parchment paper is the most satisfactory one for deciding whether a particular solution is a colloidal one. This property goes together with the various other properties dependent on surface development, although it must be admitted that it is somewhat arbitrary to fix the point at a definite dimension. Indeed, there are substances on the border line, like certain dyes, which will pass through some samples of parchment paper, but not through others, and these substances are found to possess some of the colloidal characteristics but not all.

When we are dealing with such things as gold, silica or arsenious sulphide, we know that the size of their particles can only be attained by the *aggregation* of a number of molecules; but, as we have just seen, the *single molecules* of some organic compounds, such as starch, may be of sufficient size to present properties of surface. Hæmoglobin does not pass through parchment paper, but measurements of its osmotic pressure by Hufner and Gansser (1907) have shown that it is present in solution in single molecules. How this is known will be understood after Chapter VI. on osmotic pressure has been read. In the case of salts, such as Congo-red or caseinogen in alkaline solution, which are electrolytically dissociated in solution, but of which neither ion passes through parchment paper, complications are present which will be discussed in the next chapter. It may be that the organic ion itself is sufficiently large to possess the properties of the colloidal state, or there may be aggregates of these ions formed.

THE ULTRA-MICROSCOPE

Much of the recent progress in knowledge of the colloidal state is due to the use of the ultra-microscope. This method was first described by Siedentopf and Zsigmondy in 1903. Details of the construction of the instrument would be out of place here. The reader is referred to the original paper (1903) or to the book of Zsigmondy (1905, pp. 83-97). Space for the principles only, on which it depends, can be found here.

It is a matter of common observation that dust particles, completely invisible under ordinary light, become clearly visible in a beam of sunlight. Rayleigh (1899) has shown that to make visible a particle, which is too small to be seen by the highest power of the microscope, merely requires sufficiently intense illumination. It must be remembered that these particles are smaller than the wave lengths of the visible part of the spectrum. For example, the wave length of the D line of sodium is 589 $\mu\mu$ and the limits of the visible spectrum lie roughly between 700 and 400 $\mu\mu$. Dimensions of such values are high for the particles in a colloidal solution, which may be as small as 6 $\mu\mu$, as we have seen, although this is an unusually small size. Any object smaller than half the wave length of the light by which it is illuminated cannot be seen in its true form and size owing to diffraction. Hereby is set a limit to microscopic observation. A brilliantly-illuminated dust particle in a beam of sunlight is seen as a disc, due to diffracted rays sent off from its surface, and looks much larger than it actually is.

The Faraday phenomenon in a colloidal solution is similar to that of the motes in a sunbeam. It occurred to Siedentopf and Zsigmondy that if the solution was much diluted and the beam examined by the microscope, placed perpendicularly to its track, so as not to receive the direct light, the diffraction images of the separate particles would be visible. In that form of the ultra-

microscope made for the examination of liquids, a very intense but small beam of light is projected horizontally from the sun or an arc lamp, by means of a system of condensing lenses, into the liquid contained in a small cell with a flat side towards the light and a flat top towards the observer. The track of the beam is examined from above by means of a water-immersion lens forming the objective of an ordinary microscope. If the solution contains particles, these are seen as bright discs with vigorous Brownian movement. The limit of visibility depends on the intensity of the illumination. The finest particles cannot be distinguished separately, but are indicated by a haze. Zsigmondy uses the name, "submicron," for elements seen as separate discs, although invisible in the ordinary microscope, and "amicron" for those which even the ultra-microscope can only indicate as a diffuse illumination in the track of the beam. Fig. 36 shows the course of the light rays; Fig. 37 the arrangement of the apparatus, in Zsigmondy and Bachmann's (1914) pattern, and Fig. 38 shows the illuminated field seen by the observer.

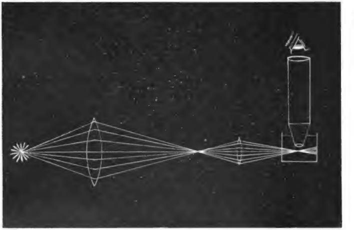


FIG. 36. DIAGRAM OF THE COURSE OF THE RAYS OF LIGHT IN THE ULTRA-MICROSCOPE.

The correct interpretation of all the phenomena seen by this method has not been arrived at as yet, and much caution must be exercised in drawing conclusions. There are one or two points which a little experience in its use with a variety of solutions has taught me, that it may be well to call attention to. It is a matter of some difficulty to obtain water that does not show a few particles, so that in the preparation of colloidal solutions foreign particles are almost unavoidable. Now these may be mistaken for the substance under examination. To take an example, a dilute solution of Congo-red, even the purest, is almost certain to show a few bright discs of light; but, on close observation with the most intense light that the apparatus can give, it will be noticed that the track is filled by a faint haze. In this case it can easily be shown that the few particles seen are not the dye itself; the addition of a little acid to the solution splits off the free dye acid and this forms a colloidal solution with comparatively large particles, so that the whole track of the beam appears densely packed with bright diffraction images. It is unnecessary to repeat that this appearance of densely-packed particles is really due to the disparity in size between the objects and their diffraction images. What is clear is that the dye salt is not resolvable into particles, it consists of "amicrons," whereas the free acid consists of "submicrons."

Again, if the refractive index of the particle approximates to that of the external liquid phase, the system is not optically heterogeneous and no opportunity for formation of images is present. This seems to be the case with certain hydrophile colloids, especially proteins.

If a given solution cannot be resolved, it must not, therefore, be assumed that it is a true solution. It may consist of particles too small to be seen by the

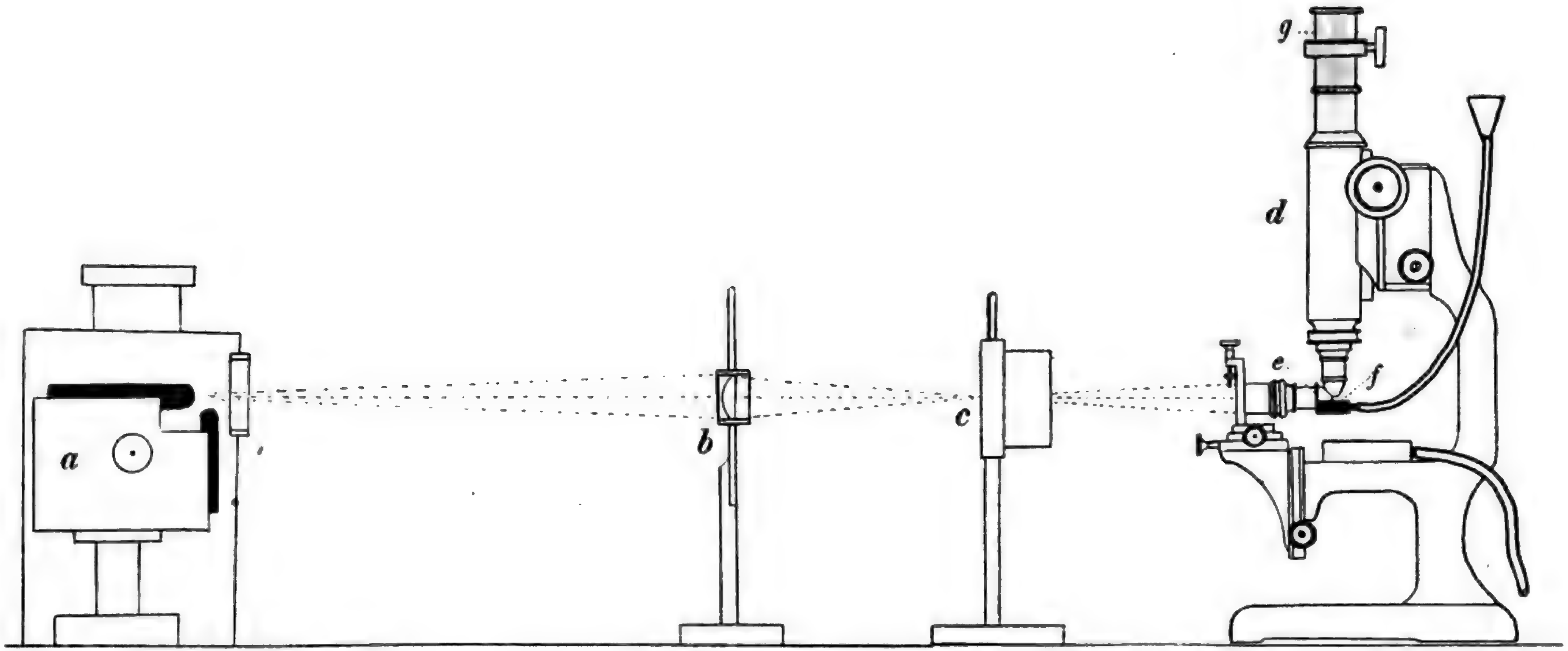


FIG. 37. ARRANGEMENT OF THE ULTRA-MICROSCOPE IN ZSIGMONDY'S LATEST FORM.

a, arc lamp. *b*, long focus condensing lens.
c, precision slit to limit the area illuminated. Turns through a right angle for the purpose of counting particles.
d, observing microscope, with condensing objective *e*, and ocular *g*, fitted with slit.
f, cell for solution, into which both the observing and condensing objectives dip. Both are water-immersion lenses.

(Zsigmondy und Bachmann, 1914. As made by Winkel and supplied by Zeiss.)

available illumination, or their refractive index may be too close to that of the surrounding medium.

In the actual instrument, as supplied by the firm of Zeiss or Winkel, for example, the crater of the positive carbon of the arc is first focussed on to an adjustable slit, whose aperture can be read off on the graduated head of the adjusting screw. This slit can be rotated through 90° for the purpose of *estimation of the actual mass of the particles* by the ingenious method of Siedentopf and Zsigmondy (Zsigmondy, 1905, pp. 93-97). The total content of the colloidal matter in a large volume of the solution is first determined by some appropriate chemical method. This solution is then diluted to a known extent, and so far that the particles seen under the ultra-microscope are sufficiently separated to be counted. By aid of a micrometer in the ocular, a known area of the field is isolated, and the number of particles in the volume corresponding to this area is counted. The depth of this portion is obtained by rotating the slit through 90°, when what was previously the depth becomes the width, and can be read off on the ocular micrometer. A simple calculation then gives the number of particles in unit volume of the original solution, and from this the mass of each is known from the total solid content of the solution.

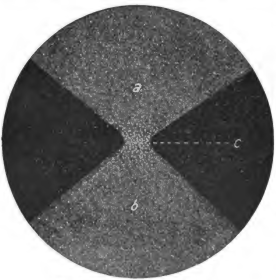


FIG. 38. ILLUMINATED FIELD AS SEEN.

The rays converge to a focus in the centre and then diverge again, *a* and *b*. Note that the greatest number of particles is rendered visible in the most brightly illuminated spot *c*. This is due to the fact that the more intense the illumination, the smaller are the particles that it is possible to observe. The particles which are too small to be seen outside the focus of the beam are obvious under the more brilliant light at this focus.

Dark Ground Illumination. — Another method, sometimes called "ultra-microscopic," which is frequently used for examination of bacteria, and is then, of course, not strictly ultra-microscopic, but can also be made to show the presence of structures invisible by the ordinary microscope, is a development of the dark ground illumination by specially constructed sub-stage condenser, introduced by Wenham in 1872. The central rays of the illuminating beam are cut out by means of a stop, and the peripheral rays are reflected by a parabolic surface so as to meet at a point in the object under examination; they cross at such an angle as to pass outside of the field of the objective in use, which only picks up light refracted, or diffracted, from structures in the preparation. The paraboloid form is chiefly used for the investigation of comparatively coarse structures, as in Fig. 6, of Spirogyra. A cardioid surface, as in the apparatus of Siedentopf, made by Zeiss, gives more brilliant illumination, and can be used for the more minute particles of colloidal solutions. This latter instrument has also been fitted, at my suggestion, with an electrical heating arrangement, so that the changes produced in colloids by heat can be followed by the eye.

The scattering of light by suspended particles has been made the basis of a method of estimation by Theodore W. Richards (1906; also Biltz, 1907). Accurate determinations of small amounts of precipitates can be made in this way. The instrument used is called, by Richards, "*Nephelometer.*"

DIALYSIS

One definition of the colloidal state is that matter in this state does not pass through such a membrane as parchment paper. The discovery of the fact is due to Graham (1861, p. 186), as well as the application of it to the separation of colloids from crystalloids by the process which he called "dialysis." The forms of apparatus which he used are shown in Fig. 39, and are in practice very effective.

I find that it is better not to allow the level of the liquid inside to rise above the upper edge of the paper, since it is difficult to make a tight joint at the lower edge of the hoop or glass bell. The sheet of paper taken should be large enough to be tied around the top of the vessel. A continuous current of water may be caused to flow through the outer vessel, but a given volume of distilled water is more effective if used in several changes of the whole volume of liquid in the outer vessel.

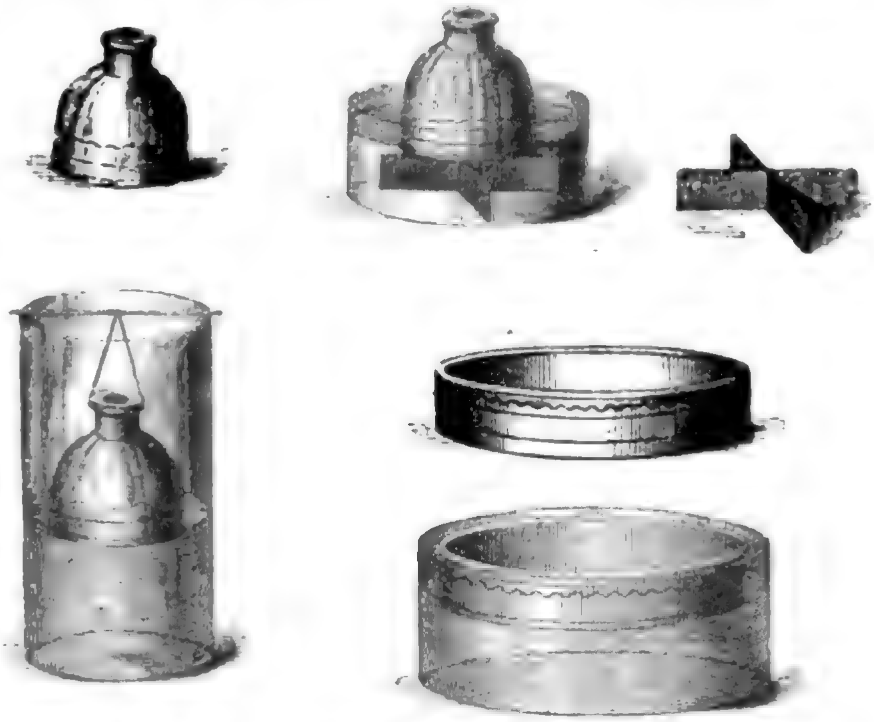


FIG. 39. VARIOUS FORMS OF DIALYSING VESSELS USED BY GRAHAM.

(Pp. 556 and 573 of his "Collected Researches.")

Crystalloids pass very rapidly through parchment paper. Graham showed that 96 per cent. of the salt content of a 2 per cent. solution of sodium chloride passed through in twenty-four hours, when the volume of the water outside was ten times that of the solution and was changed once. Dilute hydrochloric acid applied to one side of the paper reddened litmus paper on the opposite side in 5·7 seconds. A point to be remembered is that the paper itself is altered by the action of alkali, expanding more than by the action of water alone. This will affect its permeability, and, in fact, I have noticed that Congo-red, which passes very slowly through some samples of the paper, is accelerated in this process if the solution is slightly alkaline.

Other forms of dialyser will be found described in the practical handbooks, such as the article of Zunz (1912, pp. 478-485).

J. J. Abel (1913 and 1914) has applied the process of dialysis to the investigation of chemical changes occurring in the whole organism of the higher animals or to those occurring in individual organs. The blood, issuing from an artery through a canula, is made non-coagulable by the addition of small amounts of extract of the heads of leeches, run into it from a side tube, and is then caused to pass through a series of collodion tubes, immersed in warm Ringer's solution. Collodion, like parchment paper, is impermeable to colloids. After passing these tubes the blood is returned to a vein and thus is kept in continuous circulation through the dialyser. In its passage, it gives up the diffusible substances which it contains to the outer fluid, in so far as they are not already present in equal concentration therein. By sufficiently long continuation of the process, these substances pass out until they are in equal concentration in the blood and in the outer liquid. If the maximum degree of dialysis is required in a

limited time, the Ringer's solution is changed at intervals. Abel has already obtained considerable amounts of amino-acids. To investigate the changes taking place in the contents of the blood as it traverses a particular organ, the diffusate from the ingoing blood can be compared with that of the outgoing blood. The substances that have been identified as diffusing out from the blood are sugar, urea, phosphates, amylase, and amino-acids. The name of *vivi-diffusion* is given to this method by its discoverer.

ULTRA-FILTRATION

Although membranes of hydrophile colloid substances do not allow water to filter through at any perceptible rate under moderate pressures, it is possible, by the application of pressures from two to thirty atmospheres or more, to concentrate colloidal solutions and separate them from "crystalloid" admixture, by forcing the liquid phase through the membrane.

This was first done by Chas. J. Martin (1896). His filter consisted of a porous clay Chamberland candle, whose pores were filled with gelatine. This was fixed in a gun-metal case, with the nozzle projecting, and the space between the two was filled with the liquid to be filtered. A pressure of some thirty atmospheres or more, applied to the solution, caused the water and crystalloids to be driven through, while the colloids remained behind.

Bechhold (1907) modified the apparatus so that flat sheets of various membranes, differing in permeability, could be used. He also showed how to make membranes of different degrees of permeability. Some of the results obtained by this method will be referred to in Chapter V., on "Permeability of Membranes." The name of "Ultra-filter" is due to this investigator. See also the work of W. Brown (1915) and of Walpole (1915).

BROWNIAN MOVEMENT

If sand be shaken with water, and the mixture then allowed to stand, the sand rapidly falls to the bottom, leaving the water clear and free from grains. Why, then, do the particles of gold, whose density is greater than that of sand, remain suspended for an indefinite time in the colloidal state?

It will be obvious that this is, in some way, connected with their size; but there must also be forces active in preventing them from sticking together to form grains large enough to fall rapidly, as the following consideration will show. The larger the number of particles into which a given mass is divided, the greater the surface energy. Now, by the principle of Carnot and Clausius, the system strives to diminish this free energy, so that, unless prevented, the particles will aggregate together to form larger particles and sink.

In 1828 the botanist, Robert Brown (1828), noticed particles in microscopic preparations to be in a continuous state of rapid oscillatory motion; the smaller the particles, the greater the amplitude of the movement. Various suggestions were made from time to time to explain this "*Brownian*" *movement*, such as inequality of temperature, electrical charge, and so forth, but none were found to stand the test of experimental investigation.

One fact, which at once disposes of any hypothesis referring the movement to any external cause, is the complete independence of the direction of movement of two particles in close proximity to one another. That electrification has nothing to do with the phenomenon is shown by an experiment of Svedberg (1907). By gradual addition of an aluminium salt to a colloidal solution of silver, he was able, owing to facts which will be explained below, to reverse the sign of the electric charge on the silver particles, thereby passing through a stage of zero charge, without in any way diminishing the extent of the movements. A reference to Brown's paper is to be found in "Middlemarch," Book II., Chap. XVII.

It is only in recent years that it has been shown, chiefly by the work of Perrin (1908), that this movement is identical with that of the molecules of the liquid, as postulated by the kinetic theory.

In order to understand the nature of the proof, which has also important bearings on the question of the real existence of molecules, a few words are necessary on the *kinetic theory* and on the molecular basis of chemical science.

Certain difficulties in the atomic theory of Dalton, when applied to the volumes of gases taking part in reactions, were removed by accepting the law proposed by Avogadro in 1813, namely, "equal volumes of gases at the same pressure contain equal numbers of molecules." Now, if molecules have an actual existence, it follows that, in a definite volume of any gas, say one cubic millimetre, there is a certain definite number of molecules. When expressed as the number of molecules in one gram-molecule of a gas, or in 22·4 litres at standard temperature and pressure, it is known as "Avogadro's constant," and is usually designated by the letter N. It has been determined by several independent methods, and the fact that the values obtained lie very near together is, in itself, powerful evidence of the truth of the assumption on which they were calculated. A short account of these methods will be found in Perrin's monograph (1910, pp. 75-93).

Further, according to the kinetic theory of gases, these molecules although very minute have a finite size, and the space occupied by the molecule, or rather by its sphere of action, is very small compared with the space unoccupied. At all temperatures above absolute zero the molecules are in ceaseless movement. Any one molecule will travel in a certain direction until it meets another one. After collision and interchange of kinetic energy, the two molecules will rebound and travel again, but with a velocity changed in direction and in magnitude, until further collisions occur. It will be seen that when the gas is a mixture of molecules of various masses the kinetic energy of any individual molecule will vary from moment to moment, but will oscillate about the mean value. Similarly, the distance travelled between collisions will vary about a certain value, called the "mean free path."

It is interesting to remember that, although the first actual publication of the kinetic theory was made, independently, by Kroenig in 1856 and by Clausius in 1857, a complete development of the theory had been sent to the Royal Society in 1845 by J. J. Waterston. This paper, unfortunately, was not printed until 1892, in the *Philosophical Transactions*, having been found by Lord Rayleigh in the archives.

Similar statements apply to liquids, with the exception that the molecules are in such close relation that the cohesive force of attraction, the quantity a of Van der Waals' equation, about which we shall have more to say later, comes into play much more powerfully, as does also the other quantity b, representing the volume of the molecules themselves. In the case of solids, this molecular movement, due to heat, must be supposed to be confined to oscillation about a mean position. The molecules of solids do not continually change their places, as is the case with gases and liquids.

Let us now fix our attention on a particular molecule in the interior of a liquid. It will be driven hither and thither by the impact of other molecules, upwards, downwards, and so on, occasionally taking a comparatively long journey before collision with another molecule.

It can be easily shown (Perrin, 1910, p. 11) that the mean molecular kinetic energy is the same in all gases, and van't Hoff has shown that the same statement holds for dilute solutions; so that a molecule of alcohol in solution in water has the same kinetic energy as each molecule of the water. Again, the molecules of sugar in solution have the same mean energy as those of the water, as also have those of any other molecule, light or heavy, in true solution. Why, then, should we not extend the conception to aggregates of molecules, in other words, to colloidal particles? This is the starting point of Perrin's important work.

Consider first what will happen to a particle very large in comparison with the molecules of the liquid in which it is immersed. It will be bombarded on all sides by a large number of molecules, moving in all possible directions, whose resultant will be zero or very nearly so, and no movement will be perceptible. As the particles are imagined to become smaller and smaller, they will be hit by fewer and fewer molecules simultaneously, so that the forces acting on them will cease to be balanced, and the particles will be driven hither and thither just as the molecules of the liquid itself. There is thus every reason to suppose that their mean kinetic energy will also be identical with that of the molecules of the liquid or of any other molecule in solution.

Suppose next that, on this assumption, we proceed to calculate the constant of Avogadro from direct observation of the Brownian movement or of states of equilibrium due to its operation. If the values arrived at agree with those obtained in other ways, the proof is practically complete that the hypothesis is a valid one. This is what Perrin (1910) has done.

Three different methods were adopted, the exact details of which will be found in his little monograph. The first method depends on the fact that, if the Brownian movement of particles is really the same as the movement of molecules in a gas, their vertical distribution in equilibrium must follow the same law as that of the atmosphere, under the influence of gravity. In order to verify this experimentally, it was necessary to prepare suspensions of particles of a uniform size and sufficiently large for the observation to be made in the depth of a cell on the stage of the microscope. The use of the microscope was

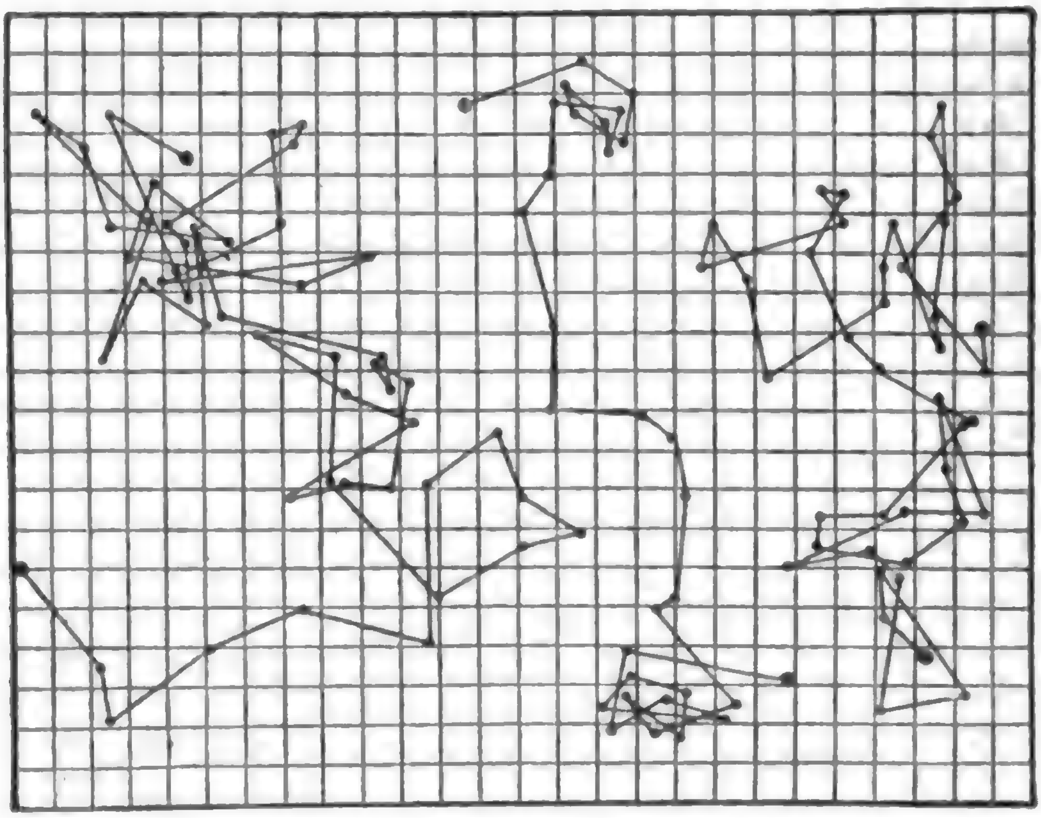


FIG. 40. BROWNIAN MOVEMENT.—Paths obtained by joining the consecutive positions of three particles of mastic at intervals of thirty seconds. They only give a feeble idea of the complexity of the real trajectories. If the positions were indicated from second to second, each of the rectilinear segments of the figure would be replaced by a polygonal contour of thirty sides, as complicated as the drawing given here.

(Perrin, 1910, p. 64 of Soddy's translation.)

necessary in order to count the particles. Gamboge and mastic were the substances used. By a process of fractional centrifugation, preparations containing particles of a uniform size were made. From these experiments, a value of $70{\cdot}5 \times 10^{22}$ was found for the number of molecules in 22·4 litres of a gas.

The second method was based on a formula of Einstein, giving the mean displacement of a particle in a given time in terms involving N, together with other values capable of experimental determination. The positions of an individual particle were mapped out at intervals of thirty seconds by the camera lucida on squared paper. Samples of three such tracings are given in Fig. 40. This figure will serve to give some idea of the complexity of the movements in question, but only a limited one, since it must be remembered that, if the position of the particle had been mapped at more frequent intervals, it would have been found that between each of the positions marked, a path fully as elaborate as the whole of the figure would have to be inserted.

These figures are also instructive as showing what complexity results from the action of apparently simple and uniform forces. The mean kinetic energy of each molecule is the same as that of other molecules, and the forces to which it is exposed might be imagined to be symmetrically distributed in the body of the liquid, and yet we obtain this apparently "chaotic" variety of movement. It is unnecessary to remark that it is not really in any way "chaotic," the impression it gives us is merely due to our inadequate methods of observation. By this second method a value of N of $71 \cdot 5 \times 10^{22}$ was obtained.

The third method depends on the fact that, when the dimensions of the particles are sufficiently large, many of the impacts of the water molecules will be directed more or less tangentially, and so cause rotation of the particles, which can be observed when these contain some distinguishing mark, as an inclusion in course of their formation. A formula, also due to Einstein, gives the possibility of another determination of N, which comes out as 65×10^{22}.

If we compare these various values with the latest and most accurate measurement by Millikan (1917), by the method of electric charge on gas ions, which gives $60 \cdot 62 \times 10^{22}$ with an uncertainty of only 0·1 per cent., we must be struck by the very close agreement, and have no hesitation in admitting the truth of the view that Brownian movement is the same thing as the molecular movement of the kinetic theory. Perrin's latest results (1911, pp. 1-2), indeed, give values still closer to the number found by Millikan.

Experiments were also made by the second method with much larger particles in 27 per cent. solution of urea in order to keep them in suspension. These gave a value for N of 78×10^{22}. Considering the small number of observations made, the agreement must be regarded as satisfactory. Since the foundation of Einstein's theorem is the assumption of equal partition of kinetic energy, and the experiments showed that particles differing in diameter 60,000 times gave the same value of N, they must be looked upon as the most weighty confirmation of the hypothesis of equal partition of kinetic energy.

It should be remembered that Ramsay (1891) advocated the view that Brownian movement is due to the impacts of molecules of the liquid against the particles, and that Ramsay and Senter (British Association Reports, 1901) concluded from the fact that the density of colloidal solutions of arsenious sulphide is the same, whether measured by the hydrometer or by weighing, that the particles of the colloid hit against the hydrometer to float it with the same energy as the molecules of the water do.

It is impossible to avoid some satisfaction that further evidence is given by Perrin's experiments, that we are not compelled to be content with equations derived from energetics, since the visible particles of these experiments behave precisely like the supposed molecules of the atomic theory. The chemist may also regard his structural formulæ with more satisfaction of their approximate resemblance to actual fact, and van't Hoff's theory of solutions is confirmed.

In connection with the illustration of the kinetic theory afforded by the Brownian movements, as pointed out above, attention may be called to the fact that theories dealing with the movement of molecules, such as the kinetic theory of gases, are essentially *statistical*, that is, they are not concerned with the actual energy possessed by an individual molecule at a given instant of time, but with the average of a very large number. If the energy of a single molecule at a given moment of time could be measured, it might be found to be a very long way off from the mean. The valuable essay by Guye (1917) on the application of the calculus of probabilities to physico-chemical and biological questions should be read.

This consideration is probably that which lies at the basis of the possibility, to which Donnan has called attention, that a living organism might appear to evade the second law of energetics. If we look upon an individual organism as a molecule in respect to the world of similar organisms, it does not seem, prima facie, altogether impossible that its activities might so far differ from the mean as to contravene the laws deduced from the general mass. But, in point of fact, we do not meet with deviations of this kind. We know, for example, that if we stimulate the vagus nerve, the heart will certainly stop, *except* some counteracting agency, such as atropine, is present, *which we can lay our finger upon and allow for, in due order.*

OTHER CONDITIONS OF STABILITY.

Although the Brownian movement is the chief cause of the permanency of the colloidal state, there are some other conditions which play a part. The *density of the medium* in which the particles are suspended will clearly have an effect,

The greater the density, the less the effective weight of the particles, hence the greater will be the buoyant effect of the bombardment by the water molecules.

The presence of an *electric charge* will also tend to prevent aggregation, on account of mutual repulsion. If by any means a number of the particles are given opposite charges to the remainder, aggregation will naturally be brought about by mutual attraction. This question will be discussed below. That the electric charge is not the sole cause of permanent suspension is shown by the fact that it can be reduced to zero, without affecting the stability, as in the experiment of Svedberg, given on page 84 above, where the Brownian movement was unaffected.

The opposing action of mechanical surface tension and electric charge has already been indicated. Lewis (1909, 3) shows how, with a given electrical charge, at a certain definite radius of the particle, the surface energy will be at a minimum, and therefore the stability at a maximum. It will be remembered that the surface tension is tangential and the electric force radial, so that it is only the radial component of the former which is opposing the electric force. This latter, however, acts inversely as the fourth power of the diameter, while the former acts inversely as the simple diameter.

The *viscosity* of the external phase should also be referred to. Increase of internal friction of the medium of suspension will increase the time taken for particles to fall under the action of gravity.

THE COLOUR OF SOME HYDROSOLS

Interesting evidence of the gradual transition from molecules to colloidal particles is afforded by the work of Svedberg (1909, 2) on the colour of gold hydrosols. With increasing dispersion, that is, more minute subdivision, the colour of the colloidal solution of gold approximates more and more to that of a gold salt in true solution, or the colour of the gold ion, supposing the anion to be colourless. The absorption in the spectrum shifts more and more towards the ultra-violet, where gold chloride possesses a characteristic absorption. Wöhler and Spengel (1910) have shown also that coarsely colloidal platinum is of a more or less violet colour, which becomes more and more like the orange colour of platinum salts as the dispersion is increased. Wo. Ostwald (1911) shows that the maximum of absorption, as a general rule, gradually passes to the *shorter* wave lengths as the particles become *smaller*, so that the colour of the solution, that is, the colour of the light *transmitted*, changes from blue or green to red and yellow. For further details, the reader is referred to the interesting article by the last named author.

The relationship between the dimensions of the particles and the wave length of the light absorbed obviously suggests effects of *resonance*, or simple relationship between the rate of vibration of the particle and that of the light absorbed.

This phenomenon of resonance enables a considerable amount of energy to be accumulated from a series of periodic impulses, each of a very minute energy, and deserves a little consideration. Suppose a pendulum with a rate of vibration of one second, reckoned as the time elapsing between the passage through any position, and the next passage in the *same* direction. If we start with such a pendulum at rest, and give it a very slight push in the plane of its vibration, and repeat this at intervals of one second, it is possible to get up a considerable amplitude of vibration; each impulse adds its effect to that of the previous ones. Unless the interval between the periodic impulses is a multiple of the time of vibration of the pendulum, only a very small amplitude, if any at all, will be obtained, since it will only occasionally happen that the impulse is delivered in the same direction in which the pendulum is moving; all other impulses will retard the movement, energy from the pendulum being given back to the body producing the periodic impulses.

This resonance process plays a large part in decomposition by light and, if we remember the rates of vibration of light and of molecules, we realise the possibility of considerable energy changes in comparatively short times. The rate of vibration of the light of the D line of sodium is, in fact, about 5×10^{14} per second.

Resonance also comes into play in the production of powerful high frequency electrical discharges, as used in electro-therapeutics, and in the action of the auditory apparatus, according to the theory of Helmholtz.

An instructive model to illustrate the phenomena of resonance has been designed by Burch (1913, p. 490).

THE ELECTRICAL CHARGE

The fact that contact surfaces between phases are usually the seat of differences of electrical potential has been referred to in the previous chapter. It is not surprising, therefore, to find that such charges play a large part in the properties of the colloidal state.

The origin of these charges is clearly, in many cases, electrolytic dissociation. Imagine a particle of silicic acid in water. This particle consists of a very great number of molecules. Silicic acid must be supposed to be not wholly insoluble in water. The outer layer of molecules will, therefore, be dissociated. $H^{\cdot}$ ions will travel off, in accordance with their great mobility, while the silicate anions, probably on account of their relative insolubility, remain as a layer on the outer surface of the particle. This particle will then have the negative charges corresponding to a large number of dissociated molecules and will behave as a multivalent anion. Similar considerations will apply to all acidic substances in the colloidal state. If basic, such as aluminium hydroxide, OH' ions will be given off, leaving a multivalent cation. Substances of the kind here described are called by Hardy (1910) "electrolytic colloids" and the huge aggregate, partially dissociated, a "pseudo-ion" or, preferably, a "colloidal ion."

When such a colloidal solution is examined by the ultra-microscope, the particles are found to be of various sizes, but, if exposed to the field between oppositely charged electrodes, they all move at the same rate. The differences of potential between them and the external water phase must therefore be the same for all. It follows that the charge must be directly proportional to their size. While a true ion, of the same chemical composition, always carries the same charge, these colloidal ions carry variable charges, although the chemical nature is unaltered. If the charge be due to surface dissociation, as described, it is natural that more ions should be produced on a large surface than on a smaller one.

Some colloids are electrolytically dissociated in water to as great a degree as many inorganic salts are. Dyes with a large molecular weight, such as Congo-red, belong to this class. The precise nature of their solutions is not yet clear, since the osmotic pressure is less than would be expected from their conductivity (see my work on Congo-red, etc., Bayliss, 1911). Salts of proteins with a strong acid or base, such as sodium caseinogenate, or globulin hydrochloride, belong to this class.

Now, Congo-red is a sodium salt and presumably, on dissociation, $Na^{\cdot}$ ions will be formed. These ions can readily pass through parchment paper, as shown by the diffusion through it of sodium chloride. But, in the presence of the colloidal anion, they are held back. How? The answer is, by electrostatic attraction. An ion cannot leave the immediate neighbourhood of an oppositely charged ion, unless much work is done in overcoming the attraction. For this reason, the $H^{\cdot}$ ions in the case of silicic acid are held in close proximity to the oppositely charged particle, forming, in fact, one component of a Helmholtz double layer. Certain important phenomena due to colloidal salts bounded by membranes are due to the same fact, as will be seen in the following chapter.

Substances like Congo-red and salts of caseinogen may be called "electrolytically dissociated" colloids, to distinguish them from the electrolytic colloids of Hardy. At the same time it may turn out that the two are essentially the same, since the large colloidal ion may really consist of aggregates of ions in both cases, although in the former, these aggregates, if present, are too small to be resolved by the ultra-microscope; the utmost that can be seen is a faint haze. Another form of such colloids is that of the "ionic micelle" of M'Bain (1920), which consists of an aggregate of ions and undissociated salt, together with molecules of water.

There are certain facts, however,. which cannot be neglected, not readily to be explained on the basis of electrolytic dissociation. Quincke (1898, p. 217) noticed that a great variety of inert substances, paper, charcoal and so on, have a negative charge in water. The similar charge on drops of petroleum (Lewis) and of aniline (Ridsdale Ellis) has already been mentioned (page 53 above), and the difficulty of explanation on a purely chemical basis was pointed out. On the

other hand, as Hardy has shown (1912, p. 632), a mere trace of a chemically-active substance, present as impurity, such as an ester or oleic acid, is sufficient to cause the spreading on water of a heavy hydrocarbon oil which, when pure, does not do so. This being so, a chemical explanation of all the above cases must not be too hastily set aside.

But also, it must not be forgotten that electrical charges can be conferred by other means than electrolytic dissociation in the usual sense. It will be sufficient to refer to the phenomena of frictional electricity. The separation of positive and negative electricity here, and the source of the electrical energy resulting, must be looked for in the mechanical work of tearing apart the constituents of the double layer, although the way the double layer itself is produced is not quite clear.

Rudge (1914) finds that dust, blown up so as to make a cloud, becomes highly charged, and that the sign of the charge depends on the chemical nature of the particles. "Acidic" substances, such as sand or molybdic acid, become negative, "basic" substances, such as coal, flour, red lead or alkaloids, become positive. The facts show that the charges of frictional electricity may, after all, be due to electrolytic dissociation.

The various phenomena connected with the electrification of gases and the action of ultra-violet light are also to be remembered. It is possibly such facts that caused Lewis to suggest an "electronic" origin for the charge in certain cases. It appears to be the point of view taken by Perrin (1904 and 1905) in his work on electrification at the surface of contact between solids and liquids. This observer found that no electrical charges are present except in ionising liquids, such as water, alcohol, etc. None was found in ether, chloroform, turpentine, etc. But, as Hardy points out (1910, p. 193), the absence of migration in a non-conductor does not necessarily prove the absence of potential difference between the phases.

Although many of the cases described by Perrin can be explained on the basis of electrolytic colloids, as stated above, it must be admitted that in such cases as charcoal, carborundum, cellulose, etc., the hypothesis of ionisation seems rather forced. It does not assist matters greatly to point to the existence of graphitic acid in the case of charcoal, while the sign of the charge on aniline is opposite to that which one would expect from electrolytic dissociation. The existence of any charge on petroleum drops is, moreover, a difficulty. How far the presence of impurities may account for some of these facts, as in Hardy's experiments on surface tension (1912, p. 632), is at present uncertain. It would be interesting to know whether Hardy's pure hydrocarbon oil, which does not spread on water, has any charge on its surface of contact with water.

An experiment of Gee and Harrison (1910, p. 46) is interesting in this connection. Alizarin (one part in 10,000) forms a colloidal solution in 2·5 per cent. alcohol, and a true solution in 50 per cent. alcohol. When a current is passed through this latter solution, no migration of the dye occurs, so that it is not ionised, nor has it any charge at all. In the colloidal solution, along with the formation of a contact surface, the particles have a charge and move in the electric field. Apparently, then, this charge cannot be due to electrolytic dissociation, since the molecules in true solution are not so dissociated. In strengths of alcohol intermediate between the above, the rates of migration of the particles showed all intermediate stages. The interpretation of this experiment, as it seems to me, is not quite simple. Owing to the lower dielectric constant of alcohol, a less charge would be expected, and, moreover, I have found that the temperature coefficient of conductivity of a suspension of well-washed alizarin in water amounts to 3·29, a value greater than that which would be given by a trace of foreign electrolyte, in fact 28 per cent. more than that of potassium chloride, and indicating some slight true solubility and electrolytic dissociation of alizarin itself (see page 77 above). If this is so, the electric charge may well be due to surface ionisation, with production of colloidal negative ions, similar to those of silicic acid. We know, indeed, that alizarin does behave as a weak acid.

On the whole, the question of the origin of the charge in certain cases requires further investigation, although it seems that Perrin's view of contact electrification has considerable justification. In the majority of cases, there is no doubt that electrolytic dissociation is the cause of the charge.

One possibility should be referred to, although the experiments of Elissafov, to be described in the next section, do not support it. If, at the contact of an "insoluble substance" with water, there is surface tension of the ordinary mechanical kind and a trace of an electrolyte be added to the water, it is conceivable that one of the ions into which the electrolyte dissociates may produce a greater diminution of surface energy than the other one. This ion would then

be concentrated at the interface, giving rise to an electrical charge. The experiments of Lachs and Michaelis (1911) show that, when a charge is already present on the surface, ions of the opposite sign are adsorbed there. If an ion decreases surface energy, it may be adsorbed and confer a charge (see p. 59 above).

Investigation on the electric charge can be made by Perrin's method (page 71 above), when the substance can be made into a plug, such as paper, sand, etc. In the case of colloidal solutions which can be dialysed free from electrolyte the method of Whetham (1893, pp. 342-345) is the best. The solution is run slowly into the bottom of the bend of a U-tube (Fig. 41) which is already half filled with distilled water or the final dialysate, which was in equilibrium with the colloidal solution, to which a little alcohol may be added in order to lower its density slightly. A sharp boundary surface is thus formed in both limbs of the tube. When electrodes, having between them a potential difference of 100-200 volts, are placed in the water, one at the top of each limb, the boundary surface rises in one limb and falls in the other, the colloidal particles being carried towards the electrode of opposite sign to themselves, and their rate of movement can be measured.

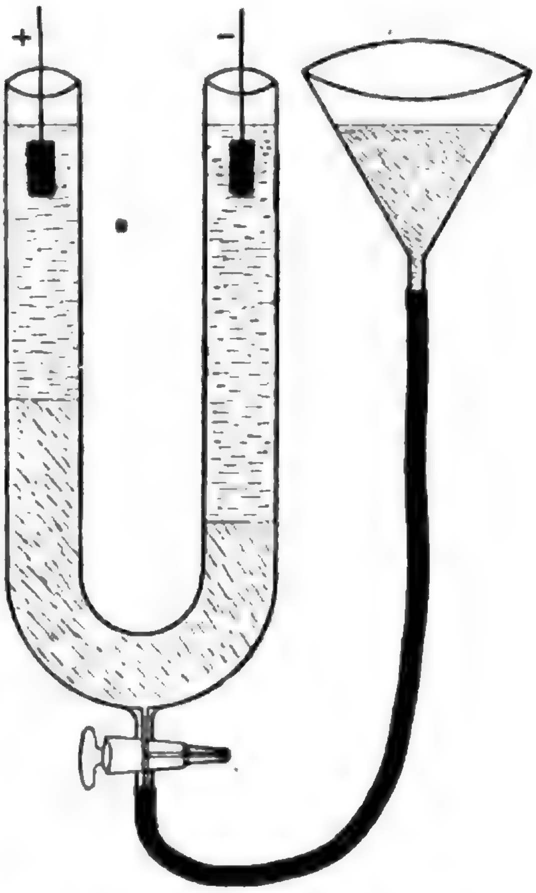


FIG. 41. APPARATUS FOR DETERMINING THE SIGN OF THE ELECTRICAL CHARGE OF COLLOIDAL PARTICLES.—(Hardy's modification of Whetham's method of measuring the migration rate of coloured ions —*Jour. Physiol.*, **33**, p. 289.) The upper part of each limb of the U-tube is filled with water, which has been dialysed into equilibrium with the diffusible electrolytes of the colloidal solution under investigation. The lower part (shaded obliquely) contains the colloidal solution. This solution has been run in slowly from the bottom under the water. Large platinum electrodes are inserted in the water at the top of each limb, and connected with a potential difference of 100-200 volts. The position of the two menisci in the figure is such as would be shown by an "electro-negative colloid" after exposure to the electric field for two hours or so.

ACTION OF ELECTROLYTES

Since many of the properties of colloidal particles depend on their electric charges, it is to be expected that the charged ions present in solutions of electrolytes would have a considerable effect upon these properties. Such is found to be the case.

The presence of H· or OH′ ions was found by Perrin (1904, p. 625) to exercise an enormous effect on the potential difference at the contact of inert solids with water. Naphthalene, for example, is electro-positive in 0·0002 molar hydrochloric acid and negative in sodium hydroxide of the same concentration. This seems to be a law which applies to the great majority of insoluble bodies, but not to all. Cellulose is negative even in 0·002 molar hydrochloric acid, though less so than in alkali. Univalent ions, other than H· and OH′, such as Na· and Cl′, have comparatively little effect. Multivalent ions, on the other hand, have a powerful effect. Suppose that a substance is in contact with a weak alkaline solution, so that it has a negative charge, the addition of a multivalent electro-positive ion will greatly reduce, annul, or even reverse the sign of the charge on the surface, and this in very low concentrations. Similarly, *mutatis mutandis*, will the presence of a multivalent electro-negative ion reduce the charge of an electro-positive surface.

What will be the effect of such alterations of charge on the suspended particles of colloidal solutions?

The fact that salts precipitate gold hydrosols was known to Faraday (1858, p. 165), and it was this action of salts which first attracted the attention of investigators. Schultze (1882) noticed that the power of various electrolytes was greatly increased by valency, indeed much beyond relation to the increased number of electric charges. Hardy (1900, i. p. 241), by more quantitative

methods, formulated a law according to which, if we call the precipitating power of a univalent ion, x, that of a bivalent ion will be x^2, and that of a trivalent one, x^3. Whetham (1899) showed that this result could be deduced from the theory of probability. Suppose that the charge of a trivalent ion is required to precipitate a certain number of colloidal particles; to obtain the same charge from bivalent ions, these particles will have to meet two instead of one; and if from univalent ions, three will be necessary. Now the chances of meeting two or three separate ions, instead of one only, are proportional to the square and cube of their concentration.

Hardy proceeded further to show (1900, 1, p. 242) that the active ion is that one whose charge is of the opposite sign to that of the colloid precipitated. He gives the following general statement: "The coagulative power of a salt is determined by the valency of one of its ions. This prepotent ion is either the negative or the positive ion according to whether the colloidal particles move down or up the potential gradient. The coagulating ion is always of the opposite electrical sign to the particle." This is known as "*Hardy's rule.*"

It may be asked, how do we know which is the active ion, since we cannot add one without the other? This is possible by taking a series of salts with the same anion or the same cation respectively. We find, for example, that potassium chloride, sulphate, and phosphate, of the same concentration in $K^{\cdot}$ ion, have the same effect on a negative colloid, say arsenious sulphide, although the valency of the anions is respectively one, two, and three. On the other hand, the chlorides of potassium, calcium, and lanthanum differ widely in their action. On a positive colloid the members of the latter series are equal, whereas the chloride, sulphate, and phosphate of the same metal are of greatly increasing potency in the order mentioned.

The following may be given as instances of electro-negative colloids: gold, platinum, arsenious sulphide, silicic acid, "insoluble" organic acids, such as caseinogen, mastic, or the free acid of Congo-red; suspensions of most powders, charcoal, kaolin, etc., are electro-negative. The hydroxides of aluminium, thorium, iron, are electro-positive.

The student is recommended to perform the following experiments on arsenious sulphide, made by passing hydrogen sulphide through a saturated solution of arsenious acid. The resulting hydrosol should be dialysed. On standing, the coarse particles will subside. Add to samples of this solution an equal volume of 0·00005 molar lanthanum sulphate, 0·0275 molar calcium chloride and 0·74 molar potassium chloride. The concentrations of the mixtures will then be as x to x^2 to x^3 in $La^{\cdot\cdot\cdot}$, $Ca^{\cdot\cdot}$ and $K^{\cdot}$ ions respectively. The precipitating powers will be found to be about equal. Experiments may also be made with varying amounts; it will be found that a concentration of $Ca^{\cdot\cdot}$ or $K^{\cdot}$ equal to that of $La^{\cdot\cdot\cdot}$ used is quite inactive, while if the concentration of $K^{\cdot}$ be taken equal to the active one of $Ca^{\cdot\cdot}$, it also will be inactive. Corresponding experiments may be made with a hydrosol of ferric hydroxide, prepared by dialysis of a strong solution of ferric chloride, which is hydrolysed, so that the free acid is gradually removed by diffusion. Potassium chloride, sulphate, and phosphate may be used. The phosphate should be neutral and may be made by mixing ten parts of molar phosphoric acid with 17·7 parts of molar sodium hydroxide and diluting to a concentration in PO_4''' ion of about 0·00057 molar (Prideaux, 1911). The corresponding solutions of sulphate and chloride may be 0·0067 and 1·35 molar in SO_4'' and Cl' ions respectively. It will be found, however, that different preparations of colloids require different concentrations for precipitation, owing to their varying degrees of dispersion, as will be shown later. It may be added that lanthanum is used as a trivalent ion on account of the fact of the minimal hydrolytic dissociation of its salts.

Before proceeding further, it is necessary to remark that the two great classes of colloids, the suspensoid or lyophobe and the emulsoid or lyophile, differ widely in their sensibility to the precipitating action of electrolytes, the former class being very sensitive, the latter comparatively insensitive. The difference, however, is merely one of degree and not fundamental, as the following facts will show. Wiegner (1910, p. 235) showed that even potassium chloride in a concentration of 2·5 millimols to 1,000 of emulsoid (olive oil and water) caused obvious aggregation when observed by the ultra-microscope. Mines (1912, p. 211) finds that egg-white is at once precipitated by a simple trivalent ion, such as $La^{\cdot\cdot\cdot}$, even in a concentration of only 0·0016 molar, although comparatively insensitive to univalent ions. Hopkins and Savory (1911, p. 213), in their investigation of the

properties of Bence-Jones protein, found that in the cold the precipitating effect of certain ions is very marked.

The mechanism of the action of electrolytes must clearly be related to the neutralisation of the electric charge on the colloidal particles by the opposite charge on the precipitating ion. It was thought at one time that the charged colloidal particles were kept in suspension by the mutual repulsion of their similar charges; in such a case, their stability should be least at the exact neutralisation point and, when excess of the precipitating electrolyte is added, so as to give the particles a new charge of the opposite sign to their original one, the condition should also be a stable one. Although in many cases this seems to be the case, in others the maximum stability has been found not to be exactly at this point. The presence of the Helmholtz double layer puts theoretical difficulties in the way of accepting the mutual repulsion of particles as being directly responsible for their permanent suspension. However this may be, it is clear that the presence of electric charges of the same sign is likely to be a hindrance to their mutual coalescence.

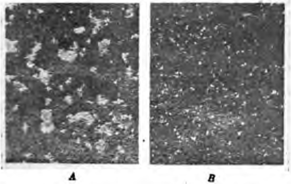


FIG. 42. AGGREGATION BY ELECTROLYTES.—Photo-micrographs of blood corpuscles of *Scyllium canicula*, suspended in half-normal sodium chloride.

A, effect of addition of 0·0008 molar cerium chloride.
B, that of 0·08 molar cerium chloride.

The dilute trivalent ion causes aggregation by reversing the sign of the charge of a part only of the corpuscles. The concentrated solution causes rapid reversal of the charge to the positive sign on all the corpuscles together.

(After Mines.)

The mechanism of the precipitation by electrolytes is well illustrated by the following experiment by Mines (1912, p. 227). The blood corpuscles of Scyllium are agglutinated (aggregated) by cerium chloride in a concentration of 0·0008 molar, as shown in Fig. 42. In a concentration of 0·08 molar they remain in suspension. Tested by their direction of migration in an electric field, the corpuscles are found to have a negative charge, when in sodium chloride of a strength corresponding to that of the blood plasma of the fish. In the strong cerium solution the charge is completely reversed, and the corpuscles are electro-positive. When the dilute solution is added, certain of them have their charge reversed before others; these positive ones will unite with negative ones, forming aggregates large enough to fall rapidly under the influence of gravity.

The fact that excess of electrolyte does actually reverse the sign of the charge on particles can be investigated by an apparatus on the plan of that of Ridsdale Ellis (1912, p. 339) which, by the use of non-polarisable electrodes, avoids the production of gas and other troublesome electrolytic disturbances.

If the charge on particles is neutralised or reversed by the adsorption of ions of opposite sign, it follows that these ions must be carried down with the precipitate. This has been shown to be the case. Linder and Picton (1895, p. 66) found that when arsenious sulphide is precipitated by barium chloride, the $Ba^{\cdot\cdot}$ ion goes down with the precipitate, while the liquid becomes acid from the hydrochloric acid set free. This $Ba^{\cdot\cdot}$ ion is held fast to the precipitate by electrostatic forces, since it cannot be removed by mere washing with water, although it can be replaced by another cation, when washed with a solution of a salt of this latter. In connection with this fact, an observation by Paine (1912, p. 62) is of interest. Colloidal copper is electro-positive (probably due to a coating of hydroxide) and the precipitating ion is naturally the anion. When this is Cl', by repeated washing of the precipitate it can be removed and the colloidal solution formed anew. When bivalent, as SO_4'', mere washing will not remove it; but, if first treated with sodium chloride in excess, so as to replace the SO_4'' by Cl', then water will restore the original colloidal solution. This illustrates the more powerful action of the bivalent ion.

In connection with this reversible coagulation, it is important to note that it has given the opportunity to Odén and Ohlon (1913) to investigate the dimensions of the aggregates before precipitation and after resuspension. Hydrosols of silver or of sulphur, after aggregation by ammonium nitrate or by sodium chloride, can be resuspended by washing with water. Investigated by the ultra-microscope, these new solutions are found to consist of particles of the same dimensions as the original ones. It would appear, therefore, that in the process of aggregation, no actual fusion takes place; otherwise it is difficult to understand how separation into particles of the same size as before could be ensured.

The carrying down of the precipitating ion with the precipitate is explained by Linder and Picton (1905, p. 1914) as due to salt formation. That this is not so is shown by quantitative relations, *e.g.*, Perrin (1905, p. 69) finds that one atom of lanthanum, as nitrate, will precipitate 425 atoms of arsenic, as sulphide. Further evidence of the same nature is given by Hopkins and Savory (1911) in the case of the Bence-Jones' protein and will be referred to under the head of proteins.

The actual number of ions carried down is of interest. Burton (1906) estimated the number of aluminium ions adsorbed by a particle of a certain preparation of colloidal silver to be 2×10^7. It is unfortunate that an aluminium salt was chosen, because these salts are hydrolytically dissociated; lanthanum should have been used. But an approximate idea of the number of atoms in a colloidal particle can be obtained by combining this value of Burton's with that of Perrin given above. One $La^{\cdot\cdot\cdot}$ ion precipitates 425 atoms of arsenic in the sulphide, so that the number of atoms in such a colloidal particle is somewhere about $425 \times 2 \times 10^7$ or $8{\cdot}5 \times 10^9$. Of course this only refers to one individual hydrosol. The dimensions of the particles vary very widely. In the case of the free acid of Congo-red, I found (1909, p. 283) by an ultra-microscopic method that the mass of each particle was approximately $2{\cdot}3 \times 10^{-11}$ mg. Taking the mass of the hydrogen atom to be $1{\cdot}6 \times 10^{-21}$ mg., that of the molecule of the acid (molecular weight $= 652$) is $1{\cdot}04 \times 10^{-18}$; so that there would be 2×10^7 molecules in each particle on the average. Each molecule contains 70 atoms, so that there would be $70 \times 2 \times 10^7$ atoms in each particle, or about one-sixth the number of those in the particle of arsenious sulphide.

Although it may be possible to represent by a chemical formula a long chain, say of 400 ferric hydroxide molecules with one of ferric chloride at the end, all united by bonds, I am unable to see what advantage is gained. It seems rather to obscure the essential nature of chemical combination, as attended by change of properties, since such colloids behave chemically as mixtures only. Moreover, these ferric hydroxide colloids must be regarded as completely hydrolysed, since it is possible to remove all the chlorine by dialysis, although great instability results. If a compound is completely hydrolysed in solution, how does it differ from a mixture? Again, it is difficult to believe that an atom of chlorine at the end of a long chain can have a chemical effect on molecules 400 places away.

If the electric charge on colloidal particles is due to surface ionisation, the greater will be this charge the finer the particles into which a given mass is divided. So that, given equal solid content of two solutions, that one which contains the smaller particles will require more precipitating electrolyte to neutralise the charge and cause aggregation. This has been found to be the case by Sven Odén (1912, p. 123) for hydrosols of sulphur and of silver. A specimen

of the former, containing particles with a diameter of 90 $\mu\mu$, required a concentration of hydrochloric acid of 1 molecule per litre. Another specimen with particles of 210 $\mu\mu$ required only 0·5 molar. When the particles were too small to be resolved by the ultra-microscope, 0·3 molar sodium chloride was required, whereas particles of 210 $\mu\mu$ only needed 0·07 molar solution of sodium chloride. It will also be noted that the smaller the particles, the greater the changes of surface energy involved in aggregation.

The fact that precipitation is due to inequality and irregular distribution of electric charges, as in the experiment of Mines related above, explains why the effect of a given amount of electrolyte depends on the suddenness with which it is added, as found by Freundlich (1903, pp. 145 and 151). If a quantity capable of precipitating, when added all at once, be added in small portions at a time, a process of acclimatisation or *tolerance* ("Gewohnung") is established and no apparent effect is produced, because the particles have all been equally affected by the electrical changes.

When the electric charge is due to surface ionisation, the mode of action of an electrolyte may be analysed further in the following way (Freundlich and Elissafov, see Elissafov, 1912, p. 411): The charge is due to the different solution tensions of the ions of the comparatively insoluble matter of which the suspended particles consist. On the surface of such a substance as glass, for example, there is a layer of ionising silicate, tending to go into true solution in the water; the $K^{\cdot}$ and $Na^{\cdot}$ ions have a great solution tension and form an outer layer; the almost insoluble, slowly diffusing, perhaps strongly adsorbed, silicate ions form an inner layer which, attached to the solid particle, give it the properties of a huge multivalent ion, the colloidal ion of Hardy. The essential difference between this and an ordinary ion is that, on account of the size of the colloidal ion, surface actions come into play, so that differences in concentration in its neighbourhood are produced by adsorption. Now, according to the law of mass action, there is a constant relation between the product of the concentrations of anion and cation on the one hand, and the concentration of the non-dissociated electrolyte on the other hand. Or, as usually expressed :—

$$(\text{anion})\ (\text{cation}) = K\ (\text{non-dissociated salt}).$$

Applied to the multivalent colloidal anion of the case before us :—

$$(\text{multivalent anion})\ (\text{cation}) = K\ (\text{non-dissociated salt}).$$

This implies that the concentration of the cation determines that of the multivalent anion, in other words, the charge on the surface, so that the cation of an electrolyte added will diminish or annul the concentration of the anion of the surface, and with it the electric charge. For further details of this point of view, the reader is referred to the paper quoted.

It is interesting to note that, according to the experiments of Dumanski (1910), substances which show all the signs of being in true solution can be converted, by the action of neutral salts, into the colloidal state. For example, solutions of molybdenum oxide showed no signs of heterogeneity under the ultra-microscope, not even a diffused illuminated cone; the depression of the freezing point also showed that the molecules present were not polymerised. On the addition of ammonium or barium chloride, or other salts, a colloidal solution was formed by coalescence of the molecules.

There is a difficulty sometimes felt with regard to the precipitation of colloids by electrolytes which must be mentioned, since it is not satisfactorily explained. When one ion of the precipitating salt is carried down with the coagulum, the other ion must be left free. To take a case, it seems that Cl' ion must be left when calcium chloride acts upon arsenious sulphide. Even if we suppose that more water is dissociated to give the increase of $H^{\cdot}$ ion shown by the acid reaction, there still remains the corresponding OH' ion to be accounted for.

EMULSOIDS

The class of colloidal solutions of most importance to the physiologist is that variously called emulsoid, lyophile, stable, or reversible. These four names, however, although in general applicable to the majority of members of the class, are not, strictly speaking, synonymous. Owing to the existence of all stages of transition, it is natural to find that certain of these characteristics may be absent

from a given substance. The word "emulsoid" indicates the liquid nature of the dispersed phase, but, since this phase may contain a greater or less percentage of water, or other solvent, with the same composition of the actual solid matter itself, as especially seen in proteins, it is clear that all degrees may exist between solid and liquid. When the dispersed phase consists of an immiscible liquid, say petroleum, the system exhibits properties approximating to those of the lyophobe class, for example, comparative sensibility to electrolytes (Lewis, 1909, i. p. 493). The fact that very minute drops of liquid have rigidity has already been pointed out, and the further fact that they are retained by the ultra-filter shows that they cannot be sufficiently distorted to be forced through apertures less than of certain dimensions, large in comparison to molecular dimensions.

Again, silicic acid is lyophile, but, after evaporation to dryness, does not again go into solution on addition of water, as gum does. It is then irreversible, contrary to most of the members of the class, which are reversible, in the sense indicated.

The designation, "stable," refers to the fact that the sensitiveness to electrolytes is much less than that of the suspended solid particles of the lyophobe class. This, again, is a matter of degree, as facts already given in the previous section (page 92) are sufficient to show. One may also refer to the fact that egg-white, a typical emulsoid, is precipitated by $La^{\cdots}$ ion in a concentration of about 0·002 molar, whereas arsenious sulphide, as we have seen, reacts to the same ion in 0·00005 molar. Remembering the ratio of activity of ions of different valency, it is not surprising that univalent ions are practically inactive on emulsoid colloids, that is, so far as their effect as charged ions is concerned.

Mines (1912, p. 211) has found a useful test for the emulsoid state. This consists in the reaction to complex trivalent ions as compared with that to simple trivalent ions. Cobalt, and some other metals, form complex salts with ammonia and an acid; these are electrolytically dissociated with the formation of a large trivalent cation, such as $Co(NH_3)_6^{\cdots}$ (luteo-cobalt) ion; emulsoids, such as egg-white, are not precipitated, even by comparatively high concentrations of this ion, up to 0·02 molar, whereas suspensoids are nearly as sensitive to it as to the simple, $La^{\cdots}$, ion.

Egg-white, coagulated by boiling, behaved in this respect as a suspensoid. As Mines points out, tea infusion contains suspensoid colloids, whereas cream is an emulsoid, so that opportunity for testing the different behaviour is ready to hand. Silicic acid seems to be an exception; although showing most of the characters of emulsoids, it is as sensitive to the complex trivalent ion as to the simple one. A fine emulsion of olive oil behaves as an emulsoid to trivalent ions.

The facts of the preceding paragraph show that *valency is not the only factor* concerned in the action of electrolytes, even in that aspect of their action connected with the electrical charge. There are two ways in which the complex ion differs from the simple one, viz., its slow rate of movement, and the less density of the charge on its greater surface. Mines (1912, p. 235) calculates the relative density on the lanthanum and luteo-cobalt ions as being in the ratio of 1·37 to 0·26, and suggests that the power of adhesion to the particle to be discharged is in relation to this fact.

The two phases of which hydrophile colloids consist differ only in the relative amount of water and solid in each. It will readily be seen, therefore, how the properties can be altered by agencies capable of *changing this distribution of water.* This point has been especially insisted on by Hatschek (1913, p. 46). If the water content of the internal phase is diminished far enough, this phase will become solid, and the system will be a suspensoid one. With large water content of the internal phase, its properties will approach to those of a liquid, and the system will be an emulsoid one. The "salting out" of proteins, etc., by high concentrations of electrolytes is due to removal of water from the internal phase, and consequent precipitation of this latter. The way in which water is present in emulsoids is regarded by Hatschek as similar to imbibition, which will be discussed later, although the possibility must not be lost sight of that more strictly chemical affinities may play a part.

When we consider the series of salts investigated by Hofmeister (1888), as regards their relative effect on the salting out of albumin, and known as the "*Hofmeister series*," no obvious reason is apparent for the different behaviour of the salts. A chemical one is excluded by the fact that the same series is found in the action on substances so different in constitution as albumin, gelatine, agar, and starch. As Hatschek (1912, p. 46) points out, the only view which co-ordinates the various phenomena is that they are all manifestations of a change in the distribution of water between the two phases; the salts of the Hofmeister series do this by their action on the compressibility of water. The solution of emulsoids is usually associated with contraction. These phenomena, in general, belong to that class called by Freundlich "*lyotropic*" (1909, pp. 54 and 412), as dependent on changes in the solvent itself. When water is the solvent, we speak of the hydration of the ions of the salt, and the changes in the equilibrium between the various molecular states of fluid water. These changes give rise, in their turn, to alterations in the internal pressure, expressed in changes of compressibility, viscosity, solubility, and so on. For further details as to the Hofmeister series, and the action of salts on emulsoids, the reader is referred to the book of Freundlich (1909, pp. 424 *seq.*).

It was known to Faraday (1858, p. 175) that the precipitating action of "salt" on gold solutions could be prevented by the addition of a trace of "jelly." Other emulsoid colloids have this action, although in different degree, and the fact serves as the basis for the "gold number" of Schulz and Zsigmondy (1903) as a characteristic of individual proteins. It seems certain that this *protection* against the action of electrolytes conferred by an emulsoid on a suspensoid is due to the deposition of a film of the former over the surface of the solid particles, thus practically converting the system into an emulsoid one. Mines (1912, p. 219), by the application of his test with complex trivalent ions, finds that a gold hydrosol protected by an emulsoid behaves in the same insensitive way as the emulsoid itself. Moreover, if the protective colloid be a protein, which has the sign of its charge easily reversed by acid, as gelatine, it will be found that the gold particles, previously insensitive to acid, have become sensitive (*ibid.*, p. 222). It can be shown by electric convection that it is not easy to reverse the sign of the charge on gold particles by acid alone; when they are coated with gelatine this is easy, although gelatine itself does not affect the sign of the charge. The adsorption of protective colloid by the surface of the gold particles is no doubt due to the lowering of surface tension thereby brought about; and the gold number varies according to the capacity in this respect.

The protective action is not necessarily complete, as I noticed in some experiments made with arsenious sulphide and with Congo-red. In these cases, actual precipitation by calcium sulphate was prevented by the addition of albumin, as in the case of gold, but if such mixtures were carefully compared with the original, it was noticed that they were somewhat more turbid. Under the ultra-microscope, the change was very obvious in the case of Congo-red. This dye, in the absence of electrolytes, is not resolvable into particles. After the addition of serum-albumin and calcium sulphate, although no precipitation occurred, as when the salt was added alone, the solution was nevertheless found to be full of very distinct, but not brilliant, particles. This effect is in agreement with the small, but not negligible, effect of salts on emulsoids.

Walpole (1913, 3) has shown that gelatine in very low concentration (1 in 100,000,000) *increases* the effect of hydrochloric acid in the aggregation of hydrosols of gold, mastic or oil. In concentrations of 10^{-6} to $10^{-4\cdot4}$ of gelatine there are two concentrations of the acid which produce aggregation, whereas, between these two, no effect is produced. In cases of aggregation due to the assistance of a "protective" colloid, reversal is obtained by the addition of alkali, and ultra-microscopic examination shows that the aggregates in the case of oil solutions consist of numbers of the original minute particles, stuck together; whereas, in the case of aggregation by hydrochloric acid in the presence of gelatine of concentration lower than 10^{-8}, the aggregates are comparatively large drops of oil. There is no change of sign of the electric charge in these cases of aggregation brought about by traces of gelatine. When concentrations of gelatine greater than $10^{-4\cdot4}$ protect from the action of acid, the sign of the charge of the particles is converted from negative to positive by the acid. For further details see Walpole's second paper (1914, 2).

A marked difference in *viscosity*, or internal friction, is shown by emulsoids and suspensoids. While that of the latter is not greatly different from that of water, the former, as a rule, have a very considerable viscosity (Freundlich, 1909,

p. 396). The various ways in which this manifests itself are only to be explained by the assumption that we have to deal with a diphasic system of two liquid phases (see Hatschek, 1913, p. 43). In contact with a solid surface, drops of the more tenacious phase adhere, and, as the fluid is forced past, these drops are deformed and torn apart. Another interesting fact, which proves the origin of the high viscosity in a two-phase nature of the system, is that mechanical deformation produces double refraction (Kundt, 1881, p. 110). In homogeneous viscous liquids, such as glycerol, strong sugar solutions, etc., this is not to be detected, whereas in gelatine, even of 0·01 per cent., double refraction can be produced by mechanical stress, which places the dispersed phase in a state of asymmetrical tension.

The viscosity of emulsoids has a high temperature coefficient.

There are two conditions very frequently met with in emulsoids, the phenomena of gelatinisation and those due to imbibition of water or other solvent. The following two sections deal briefly with these.

GELS

When a gelatine solution, which is a freely-flowing liquid at temperatures above 20°-25°, is cooled, it "sets" to a substance having the property of preserving the shape into which it is trimmed. It has, also, elasticity of form, so that, within limits, it returns to its original form after distortion. What has taken place? In speaking of the action of fixing solutions on protoplasm, the experiments of Hardy (1900, 2) were referred to. This investigator showed that gelatine, when simply cooled and unacted on by reagents, required an enormous pressure to squeeze out any of the water which it contained. This fact means that the water no longer forms a continuous phase, but must be enclosed in vesicles composed of the more solid phase, so that, to escape, the water must pass *through* gelatine. Fig. 15, B (p. 14), represents diagrammatically the state of affairs, if we regard the black as gelatine (containing water), and the white the liquid phase, that is, dilute solution of gelatine. From what we have learnt above as to the nature of emulsoids, it is clear that the word "solid" phase, used in describing the phenomena, must be understood as "relatively more solid" phase.

From Hardy's work (1900, 2) it appears that the first sign of commencing gelation is a change of the system from a micro-heterogeneous one to a more coarsely heterogeneous one, so that drops of the dispersed phase separate. It is interesting to note the proof afforded by this fact of the liquid nature of the internal phase of an emulsoid, since only a liquid could form drops. The further fate of the drops depends on the concentration of the solution. In very dilute solution, the droplets remain sufficiently small to become a permanent dispersed phase, freely movable and, in fact, showing Brownian movement. When the solution is more concentrated, the droplets join together to form a network or similar kind of structure, but the watery phase is still continuous. When still more concentrated, the droplets which separate can be seen by their refraction to consist of the watery phase, so that the more solid phase has now become the continuous or external one, while the more liquid one is the internal or dispersed phase.

The change described above is a reversible one, and is important as illustrating the kind of phenomena which may occur isothermally in a complex system of emulsoids, such as living protoplasm.

The composition of the two phases of a colloidal system, as not being qualitatively, but merely quantitatively, different, is well seen in the following figures (Hardy, 1900, 2, p. 257) from a case of a ternary mixture of gelatine, alcohol, and water. The numbers represent grams of gelatine per 100 c.c. of the gelatine solution at 15°.

Total Mixture.	Internal Phase.	External Phase.
6·7	17·0	2·0
13·5	18·0	5·5
36·5	8·5	40·0

The work of Bachmann (1912) and of Zsigmondy (1913) on the formation of gels, as observed with the ultra-microscope, is of interest. Most of the work was done with pure soaps and is illustrated by Fig. 43. It is well known that a fairly strong hot solution of sodium or potassium stearate or palmitate sets to a more or less transparent, tenacious jelly, when it cools. This usually changes later into an opaque, white, friable mass. The former corresponds to a fine felt-work, as seen under the ultra-microscope (B and F of the figure); while the latter is obviously crystalline (D in the figure). The structure of the gel, as first formed, shows a strongly polarised cone of light and is, therefore, of an extremely fine degree of heterogeneity, much finer than the foam structures described by Bütschli. As cooling proceeds, the particles become larger, Brownian movement is easily seen (A). These particles continue to increase in number, obstruct one another in movements, and suddenly form threads, which are said to have a "crystalline"

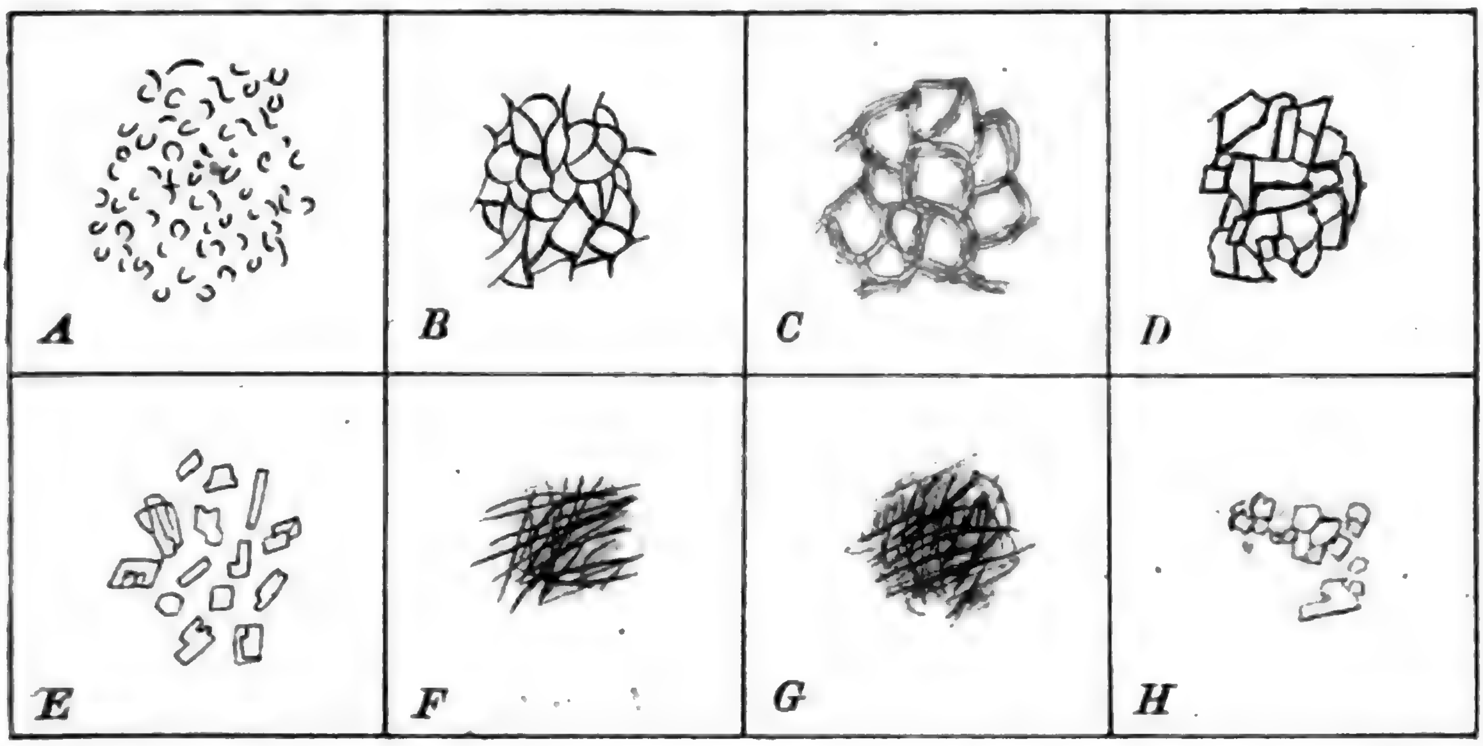


FIG. 43. DIAGRAMS OF ULTRA-MICROSCOPIC APPEARANCE OF SOAP GELS IN PROCESS OF FORMATION.

A, B, C, D, E, Stages of gelation and crystallisation of 5 per cent. potassium stearate in water. Enlarged about 200-300 times.

F, Jelly of 10 per cent. sodium oleate. Felt-work obtained by dissolving away the finer threads. Cardioid condenser. Magnified about 140 times.

G, Jelly of 5 per cent. sodium palmitate. Needle-like fibres.

H, Crystals of potassium stearate from watery solution of about 10 per cent. Final state. Magnified about 300 times.

(After Bachmann.)

appearance (see F and G), and result in the production of a felt-work. After a time, this felt-work changes into distinct separate crystals (E and H). Whether the first particles are to be regarded as "micellæ," in Nägeli's sense, that is, as aggregates with crystalline properties, is a matter for argument. It is clear, however, that the vectorial forces, which ultimately result in the formation of distinct crystals, must be always present, but apparently require time for action. The ultra-microscopic particles, probably of a crystalline form, at first separate out arranged in threads and networks. See also Bradford (1919) and M'Bain (1920).

An important point in regard to the nature of the two phases is that Bachmann found that the "inter-micellar" fluid, in the case of a gel of 1 per cent. sodium palmitate, contained 0·06 per cent. of the salt.

IMBIBITION

Many emulsoids, after being dried, are capable of taking up again large quantities of water, without actually forming liquid solutions, such as ordinary hygroscopic substances, calcium chloride, for example, do.

Most parts of plants and animals exhibit this property to a greater or less degree. The stalk of the sea-weed, *Laminaria*, increases enormously in volume under the conditions mentioned and has been made use of in surgical practice.

The greater part of the experimental work on imbibition has been done on gelatine, a considerable amount also on starch.

Perhaps the most striking thing about the phenomenon is the great pressure exerted in the process of swelling, or conversely, required to express water after it has been taken up. Laminaria, under a pressure of 42 atmospheres, was found by Reinke (1879) to be able still to take up 16 per cent. of water.

In all these processes, it is important to remember that the total volume, gel plus water, is *less* after swelling, although the volume of the gel itself increases so much. In order to compress water to the extent implied in the total change of volume, a pressure of some 300 atmospheres is necessary, so that it is plain that heat must be evolved in the process of imbibition.

This compression of water can be demonstrated in the following way, due to R. du Bois-Reymond (1913). Pieces of the dried material, such as *Laminaria*, are attached to the submerged part of a hydrometer, and the scale adjusted to a convenient point by addition of weights. As the material swells, the hydrometer sinks, showing that the water which has become part of the imbibition system has increased in density. Of course, the temperature must be kept constant.

Much work has been done on the effect of *electrolytes* on the swelling of emulsoids, especially of proteins. The most striking effect is that of acid and of alkali. Spiro (1904, p. 276) showed that either of these greatly increases the amount of water taken up by gelatine, and Chiari (1911) found that, when carefully purified, gelatine is sensitive to very small differences in $H^{\cdot}$ ion concentration, so that the difference between ordinary distilled water and that distilled out of contact with carbon dioxide may be detected. The explanation of this phenomenon, as given by Pauli (1912, p. 262), is that electrolytically dissociated salts of protein are formed by acid and by alkali, and the swelling is due to the affinity for water of the protein ion. This view will be discussed under the head of proteins.

A theory of *œdema* has been propounded by Martin Fischer (1910) on the basis of the action of acids on the swelling of proteins. The tissue colloids are supposed to take up water under the influence of increased acid reaction of the blood. Although the possibility of such effects must not be forgotten, they will not easily explain the actual presence of *liquid* in dropsical tissues; a fine canula or hollow needle inserted into such tissues allows a slow stream of fluid to drop from the end, and it is well known that œdema passes from one part of the body to another in obedience to gravity. Moreover, so far as I am aware, M. Fischer has not actually shown a change in $H^{\cdot}$ ion concentration in the blood sufficiently large to account for the effect. As we shall see in Chapter VII., the chemical composition of blood is such as to form an extremely efficient arrangement for keeping the reaction constant. And again, this delicate sensibility to change of $H^{\cdot}$ ion concentration shown by gelatine is only manifested in the absence of neutral salts, a condition not met with in living organisms.

The work of Siebeck (1912, p. 467) on kidney cells, and of Beutner (1913, p. 224) on muscle, lead them to the conclusion that the increase of size, occurring in certain solutions, is due to osmotic taking up of water, rather than to an imbibition process, and that acid or alkaline reaction has no effect unless the cells are permanently injured. Moreover, the action of neutral salts on the volume of cells is in proportion to their molecular concentration only, whereas the effect on imbibition is different according to the chemical nature of the salt, even when in equi-molecular concentrations.

Hofmeister (1888), in fact, found the action of *neutral salts* on the process of imbibition to follow the same series as that already mentioned in the case of "salting out." The relation of this series to the properties of the solvent has been indicated on page 97 above. Samec (1911, p. 156) calls attention to the fact that, parallel to the favouring effect exerted by the anions of the Hofmeister series on the imbibition of water by starch, there runs a set of

physico-chemical properties of the salt solutions themselves. These are, rate of diffusion and compressibility, which increase with the favouring action, while surface tension, internal friction, electrical conductivity, diminution of solubility of other solutes, maximum density, effect on catalysis of esters, inversion of cane-sugar by acids, and dissociation of weak acids are properties which decrease along with increase of favouring action.

At first sight it would seem natural to connect these various phenomena with hydration of the respective crystalloids in solution. A part of the solvent is held in this way in the region of the solute, so that any process in which water is concerned would pursue a different course in presence of crystalloids than in their absence, and, in general, the change would be of the same kind as that caused by increase in concentration. There seems, however, to be some additional factor, because there are some crystalloids whose solutions produce *more* swelling than pure water does. The suggestion is made by Samec (p. 157) that adsorption of the crystalloid takes place on the surface of the gel elements and that the adsorbed, highly hydrated, substance brings water into more intimate contact with the colloid. The fact that any particular ion has precisely the same effect on a protein and on starch, as pointed out by Samec (1911, p. 154), shows that the formation of chemical compounds does not play any important part. Further information as to the behaviour of gelatine in various solutions in water, may be found in the paper by Ehrenberg (1913).

As to the *nature of the process* itself, Posnyak (1912, p. 154) calls attention to three possibilities:—

1. Condensation of water on the surface of the elementary particles of the gel, leading to filling up of the capillary spaces between them, while the particles themselves remain unchanged in size.

2. Simple solution of the liquid in the substance of the particles, which thereby change their size, density, etc.

3. Both processes take place. This is regarded by Posnyak as the most probable one theoretically, although his experiments on the influence of pressure on the liquid content of a gel speak more in favour of the first. He finds, in fact, that the content in solid (c) of such gels as india-rubber and gelatine is related to the pressure (P) by the formula:—

$$P = Ac^k$$

where A and k are constants. A varies considerably from gel to gel and from liquid to liquid, while k has always the same value ($=3$). This latter fact is difficult to explain on the basis of a solution of the liquid in the colloid substance and consequent change in its properties. According to Zsigmondy (1913) the lowering of vapour pressure in the imbibition of water by silica gels is due to the formation of a concave meniscus, not to formation of hydrates. Imbibition is the filling of hollow spaces in this case, not the taking up of water into actual substance.

The similarity of Posnyak's equation to the simple form of the expression for adsorption is obvious. It would seem also to be more advantageous for rapid changes in the distribution of water, such as are required in physiological activities, that the water should be on the surface rather than inside the substance of the colloid. As Posnyak suggests, it is probable that the relative share taken by the two kinds of process differs according to the amount of water available.

Some experiments which I have recently had occasion to make favour this suggestion. Gelatine is sometimes used to remove water from alcohol that is nearly absolute, say 90 to 95 per cent. Of course, it is useless for this purpose unless thoroughly dried first. I found that it does remove water from 90 per cent. alcohol, so that this becomes stronger, but, to my surprise, no increase in volume of the gelatine was to be detected, although the amount of water removed from the alcohol was sufficient to be detected easily. The gelatine also appeared to be just as hard and horny as when put in. The only explanation seems to be that, in order to determine the volume after immersion in alcohol, the pieces were allowed to dry for about a minute in air; the liquid alcohol evaporated from the surface in this process, and apparently the water concentrated on the surface passed off with the alcohol, a phenomenon that could not have taken place in so short a time if water had penetrated into the substance of the gelatine.

The facts above described will be found in later pages to have a bearing on the action of enzymes.

PROTEINS

In many respects proteins are the most important members of the emulsoid class and, at the same time, the most difficult to treat in a satisfactory way. This difficulty is mainly due to the fact that the phenomena presented by them can be, for the most part, described from two different points of view, from that of pure structural chemistry and from the physico-chemical standpoint of colloidal chemistry.

Take, for example, the common test for the presence of a protein in solution, that with potassium ferrocyanide and acetic acid. Potassium ferrocyanide alone, in low concentration, does not give a precipitate, but such appears when the solution is made acid with acetic acid. This may be explained by saying that the compound of protein with ferrocyanide is soluble in neutral or alkaline solution, insoluble in acid. Or by saying that the negative ferrocyanic ion has no precipitating action on an electro-negative colloid, as protein is in neutral or alkaline solution, but becomes a powerful one, as a quadrivalent ion, when the colloid is made positive by the $H^{\cdot}$ ion of an acid.

Now these two points of view are not to be regarded as antagonistic or mutually exclusive. As Perrin remarks (1905, p. 110), with respect to the fact that change of electrification is accompanied by change in the composition of the double layer and, therefore, in the composition of the colloid as given by chemical analysis, "physical and chemical variations are here two aspects of one and the same phenomenon."

On the other hand, there are certain physical properties not necessarily involved in the chemical description of proteins, which must play a part in their behaviour. Since they do not diffuse through parchment paper, we know that they are large enough to exhibit the properties of matter in mass, the most characteristic being those connected with the possession of surface. This involves electrical relations differing from those of simple electrolytes, and so forth.

When we find a book, "The Physical Chemistry of the Proteins," by J. Brailsford Robertson, 1912, which professes to treat the whole subject without reference to any of the conceptions which the modern development of the theory of the colloidal state has introduced, we cannot but agree with the reviewer (W. O.) in *Zeitsch. f. physik. Chemie*, **81**, 508, whose remarks will serve to call attention to the facts to be taken into consideration in an adequate treatment. "But in so far as the colloidal, that is non-homogeneous, character of protein compounds has been proved experimentally beyond all doubt, it appears to me (the reviewer) that, in the intentional laying aside of this fact, an error of method is committed, an error which brings with it considerable danger of laying more weight on a particular interpretation of facts, in themselves correctly observed, than is desirable in the interests of science. This danger is all the more serious when the author is one who is able to manipulate, with considerable skill and corresponding predilection, complex mathematical formulæ, and by that means is able to introduce as many variables into the theoretical treatment of his problems as satisfactory agreement with experimental results requires. Owing to the complex and changeable nature of the substances in question, a completely exact agreement between measurement and calculation can never be expected, and, for this reason, the widest possibilities for theoretical presentation offer themselves."

With reference to the two theories, the purely electro-chemical and the colloidal, where the conditions at finite boundary surfaces are taken into account, the same reviewer says, "The two are, in point of fact, nowhere and never in opposition, but are merely different stages in the complete analysis of the physico-chemical phenomena."

An illustration due to A. W. Stewart (*Chemical World*, **2**, 53) will serve to show the misleading effect of a one-sided consideration. "Suppose that we gave two specimens, a diamond and some graphite, to be examined by purely chemical methods on the one hand, and by purely physical methods on the other. The chemist, relying on his analysis, would declare them to be identical, consisting, as they do, of carbon. The physicist, on the other hand, from an examination of colour, density, form, etc., would pronounce them to be different from one another. Both would be right, but each in possession of half the truth only." If we wanted to cut glass, it would not be of any help to be told that the chemical composition of diamond and graphite is the same. On the other hand, suppose that we wanted to prepare carbon dioxide by burning in oxygen, either would serve, although we should hardly choose the diamond for the purpose. Should the electronic theory of the constitution of atoms be correct, reconciliation between the chemical and physical points of view will presumably be found in molecular physics.

In order to indicate the kind of problems which confront us in the study of proteins, the work of Hopkins and Savory (1911, p. 249) on Bence-Jones' protein, may be referred to. This substance is found in the urine in certain disorders of metabolism, and is characterised by its peculiar behaviour on heating.

In the absence of salts, it coagulates on heating to about 50°, and becomes an irreversible or suspensoid colloid. If neutral salts are present, the coagulum is redissolved on boiling, owing, perhaps, to the formation of a chemical compound with salts, although, as we shall see later, this interpretation is rather questionable. If the suspensoid particles are given a positive or negative charge by traces of acid or alkali, the precipitating effect of electrolytes comes into play, and the anion or cation becomes prepotent, according to Hardy's rule. We have then a complex state of antagonistic effects. There are two relations to be taken into account, one between the salt as a whole and the protein molecule, perhaps a chemical one, although the lyotropic effects described in the preceding section must not be forgotten, the other relation, a physico-chemical, colloidal or electrical one between the particle (*qua* particle) and the ions of the salt as carriers of electric charges. In no other way can the experimental facts be satisfactorily explained.

A few words are requisite at this stage as to the chemical nature of proteins, so far as to make intelligible the way in which it intervenes in their colloidal reactions. A more complete account will be found in Chapter IX. It has been shown, mainly by the work of Emil Fischer ("Collected Papers," 1906), that these substances are formed by the condensation of a number of molecules of various amino-acids. Now the amino-acids are characterised by the presence of one or more NH_2 groups, giving them basic properties, and one or more carboxyl groups, giving them acidic properties. Alanine, or amino-propionic acid, is

$$\begin{array}{l} CH_3{-}\underset{\displaystyle NH_2}{\underset{|}{CH}}{-}COOH \end{array}$$

They belong, therefore, to the class of electrolytes called by Bredig (1899) amphoteric, behaving towards strong bases as acids, and towards strong acids as bases. When the COOH and NH_2 groups are equal in number, as in alanine, the amino-acid is very nearly equally strong as a base and an acid, and is therefore practically neutral in reaction, actually very faintly acid. If the NH_2 groups are in excess, as in the diamino-monocarboxylic acid, lysine, the substance becomes a fairly strong base; while, if the carboxyl groups are in excess, as in the mono-amino-dicarboxylic acid, aspartic acid, we have a fairly strong acid. These acids are capable of combining together by the COOH of one uniting with the NH_2 of another, with elimination of water, thus:—

$$-CO\boxed{OH \quad H}HN- \text{ becomes } -CO-HN-$$

There are always some NH_2 and some COOH groups left uncombined, and according to the relative number of these, the resulting protein or polypeptide will have the properties either of a base, a neutral substance, or an acid. This brief sketch will suffice for our present purpose, although it must be remembered that some of the constituent amino-acids are complex compounds containing aromatic, pyrrol, iminazol, etc., groups.

When combined with base or acid, say sodium or hydrochloric acid, an amino-acid forms a salt, thus alanine becomes sodium amino-propionate or alanine hydrochloride respectively:—

$$CH_3{-}\underset{\displaystyle NH_2}{\underset{|}{CH}}{-}COONa \qquad\qquad CH_3{-}\underset{\displaystyle NH_2HCl}{\underset{|}{CH}}{-}COOH$$

Similarly, the free NH_2 and COOH groups of the protein can react with acid or base to form a salt.

Now, like all salts, these salts of proteins are electrolytically dissociated in solution, the sodium salt of globulin, for example, partially dissociates into $Na^{\cdot}$ and a large organic anion, which has the properties of the colloidal state. The hydrochloride dissociates into Cl' and a large colloidal organic cation. We see thus how, by direct chemical means, we can obtain the same protein with a negative or a positive charge. It appears, also, that these colloidal ions are very ready to form aggregates, as the simple, insoluble, inorganic salts, such as

arsenious sulphide, do. It remains as yet uncertain whether many of the complex proteins, occurring naturally, are not aggregates of various simpler ones, united by means not strictly chemical.

As remarked above, proteins may be either weak acids or weak bases. In the former case, owing to the preponderance in number of $H^{\cdot}$ ions given off, the colloidal ion is left negative, just as silicic acid is; in the latter case, the protein particle will be positive, as it gives off rapidly moving OH' ions. If the protein is an aggregate, it is clear that dissociation will occur in the case of those molecules on the surface only, and the colloidal ion will be more bulky than if the protein is in single molecules.

It is of interest to record the fact that crystals of leucine, a simple amino-acid, suspended in their own saturated solution, have a small negative charge, as would be expected from the slightly more acidic than basic character of leucine itself. When a solution of leucine is made acid, there is a deposition of the solute on the cathode, when a current is passed through the solution, and vice versa when made alkaline. Thus it behaves in the same way as a protein under the same conditions.

On addition of small amounts of a strong acid to a solution of a very weak acid, by the law of mass action the dissociation of the weak acid is practically abolished, so that its molecules are almost entirely present as neutral uncharged elements. The same thing happens when a strong acid is added to a protein solution. But owing to the amino-acid nature of the latter, the effect in question is replaced by formation of a salt when the quantity of acid added is increased; the acid then combines with the basic groups of the amino-acid. At a particular concentration of acid, therefore, the protein exists with a maximum of electrically-neutral molecules. This is the *isoelectric point*, which varies with different proteins, according to the degree of their acidic properties.

Now it is found experimentally that the lyophile character varies greatly according to the presence or absence of the electric charge, *i.e.*, whether the protein is in the form of an ion or otherwise (Pauli, 1912, p. 226). The increase of hydration implied in this, goes with increase of properties such as viscosity, imbibition, solubility, osmotic pressure, difficulty of coagulation by alcohol and heat, surface tension, and rotation of polarised light. The importance of the distribution of the solvent between the phases of a colloidal system has been emphasised by Hatschek, as already mentioned, and we see now how the effect of acid and of alkali in increasing the water content of the dispersed phase may be explained by the production of protein ions. As will be shown in more detail in Chapter VIII., ions are usually associated with a considerable number of molecules of the solvent.

The action of *neutral salts* is not so simple. The example of the Bence-Jones' protein, given previously, points to a double action, if not a triple one.

The effect of salts in large concentration in removing water from the internal phase, and thus producing what is known as "salting out," has been described under the head of emulsoids above (page 97). The precipitating power of salts follows the "Hofmeister series" and it is important to note that certain properties of water, such as surface tension, viscosity, compressibility, are affected in the same order, so that it is to be supposed that the action of salts in removing water is exerted rather on the water itself than on the protein (Pauli, 1912, p. 238).

The same series was found by Rothmund, independently (*Zeitsch. f. physik. Chem.*, **33**, 401), to apply to the case of the solubility of phenylthiocarbamide. Schryver (1910) brings the phenomena into relationship with the effect of the salts on the surface tension of water.

Whether there is actual chemical union between proteins and neutral salts is a matter of some dispute. It has been suggested that reaction may occur in such a way that both potassium and chlorine, for example, may join on to the nitrogen of the NH_2 group, as H and Cl do. Another possibility is that the K may unite with COOH in the usual way, while the Cl joins to the NH_2, with the aid of the H displaced from COOH. These suggestions do not seem very probable from the chemical point of view, although, of course, not impossible. Until recently, no direct evidence had been brought forward in favour of combinations of a chemical kind, but Pfeiffer and Modelski (1912) state that they have obtained crystalline

salts of glycocoll with calcium chloride and lithium chloride, although none could be obtained with potassium chloride. A fact which also makes the evidence rather uncertain is that, in order to maintain constant composition in successive re-crystallisation, it was necessary to add dilute acetic acid. If recrystallised from water, no constant composition was shown.

I have repeated some of these experiments, but have been unable to get crystals of constant composition, even using acetic acid, and have been compelled to conclude that the preparations of Pfeiffer and Modelski consisted of mixed crystals, although it is difficult to account for their results being in accordance with these required by the chemical formulæ. I found that, unless the solutions were very highly concentrated, the pure amino-acid, both in the case of glycocoll and of leucine, crystallised out first and that it was not till the mixture was evaporated nearly to dryness, or by the addition of alcohol, that the neutral salt came down also. Further evidence on this point is therefore necessary.

On the other hand, there is certain evidence that salts are adsorbed by proteins. Such effects as those on the temperature of coagulation are found to be expressed by a formula similar to that of adsorption, and not by stoichiometrical relations. The amount of salt attached to the protein particle is found to be in certain proportion to that free in the external phase. Pauli (1912, p. 231) also points out that there is evidence that a surface action is in question, in that the viscosity of protein solutions is always lowered by the addition of a salt. This implies that the surface of contact is changed from one between albumin and water to one between salt and water.

If salts are adsorbed by protein, it is to be expected that, in the case of comparatively insoluble salts, a considerable difference would be found in their apparent solubility in water and in protein solutions. This has been found to be the case, by Pauli and Samec (1909, p. 241), for calcium sulphate, phosphate and carbonate, silicic acid and uric acid. The amount of very soluble salts adsorbed would be too small a percentage of the total solubility to be detected.

Some experiments made by myself (1906, p. 182) on the rate of removal of salts from gelatine by water, also point to the adsorption nature of the union, as also other experiments on the taking up of salts.

A further fact in connection with the question before us is that measurements of electrical conductivity show no effects of neutral salts similar to those which are so obvious where we know that true chemical reaction takes place, viz., with strong acids and alkalies.

The experiments of Bugarsky and Liebermann (1898, pp. 68, 72) show that no combination occurs between proteins and neutral salts, whereas it does between proteins and strong acids or alkalies. (See also page 221 below.)

Chemical combination between neutral salts and proteins is, then, very doubtful and cannot be used in explanation of observed facts unless directly proved to take place.

The fact that the effect of anions on the imbibition of water by starch and by albumin is identical, as shown by Samec (1911, p. 154), is difficult to understand on the assumption of a chemical union.

That there are relationships of an *electrical* nature between proteins and ions, apart from effects on the solvent or chemical reactions, is shown especially by the work of Mines (1912, p. 217) with regard to the action of various ions on emulsoids, inclusive of proteins. There is, in fact, an action similar to that on suspensoids or hydrophobe colloids. This latter we have already seen to be due to electrical charges as such and it is natural to look for similar effects in the case of proteins. Perhaps the most striking evidence in this connection is the behaviour of the heart muscle, which will be better discussed in Chapter VII., under the action of electrolytes in general. For the present, we may note that the heart of the dogfish is 10,000 times more sensitive to various simple trivalent ions than to the bivalent ion $Mg^{\cdot\cdot}$ (p. 216). This extraordinary disparity between the effects of two ions, not very different chemically, but whose electric charges are as 3 to 2, shows distinctly that electrolytes have an effect on proteins or other emulsoids in addition to the possible formation of salts, and that this effect is in relation to their electric charges. This again being due to the adsorption by a surface of ions of opposite charge to its own, will clearly depend on the sign and amount of the charge of the protein particle; in water this charge is small as a rule, but in acid or alkali, by the increased production of colloidal ions, the charge will be

greater, and, as we have seen in the case of the Bence-Jones' protein, the action of salts as ions on particles becomes more marked.

Silk is a protein and forms a convenient means of testing some of the relations of these substances to electrolytes. In pure water, it has a slight negative charge, due, no doubt, to its acidic function exceeding its basic one. As an electro-negative colloid, it is especially sensitive to the action of cations, so far as concerns all properties depending on its charge. I have recently tested its behaviour towards a colloidal acid, that of Congo-red, with which it, as a potential base, is capable of forming a salt of the usual red colour, being dyed, in fact. Now both the silk and the colloidal acid are negative, so that very little adsorption takes place, unless we reverse the sign of the charge on the silk by the addition of cations. Calcium sulphate, of very low concentration, was used for the purpose. The effect of the electrolyte was the same as in the case of filter paper as described on page 58 above. The dye was adsorbed by the silk, which was thus dyed blue, the colour of the free acid, like the adsorption compound of thorium hydroxide with the same acid. On heating, chemical reaction occurred, with the formation of a red salt of silk protein. The interest of this experiment is that it shows the intervention of electric forces in addition to the purely chemical ones. Leucine, suspended in its saturated solution, also forms a blue adsorption compound with the Congo-red acid, which becomes a red salt on warming.

The natural proteins are, as we have seen, comparatively insensitive to the action of neutral salts. Certain of them, however, known as albumins and globulins, are capable of a change, called "*denaturation*," by which they approximate to the suspensoid class, in so far as becoming more sensitive to the action of salts, although their high viscosity and low surface tension shows them to be also hydrophile. A familiar instance of "denaturation" is the effect of boiling water on white of egg. What precisely happens is as yet unknown, although the work of Hardy (1899, i. p. 182) and of Chick and Martin (1912) has thrown much light on the process. Hardy showed that in the coagulation of egg-white by heat there are two distinct stages: (1) denaturation, by which the protein becomes precipitable by salts, according to the same law of valency as the inorganic suspensoids, and (2) the agglutination of the denaturated particles by electrolytes, if present.

As we saw in the case of blood corpuscles acted on by cerium salt, if the concentration of the $Ce^{\cdots}$ ions be large, the sign of the charge is reversed on all the corpuscles together, and redispersion takes place. Similarly, dispersion of protein particles by salts can occur. When all particles are equally charged, although of an opposite sign to their original one, mutual repulsion ensues, while dispersion is also assisted by the lowering of surface tension which is the result of the increased charge. Chick and Martin (1912, p. 293) call attention to the relation of the facility with which weak acid or alkali causes redispersion of the heat coagulum of a protein to the nature of the aggregated precipitate. In a loosely agglutinated mass, each particle is sufficiently distinct to carry its own charge, whereas when the particles are closely packed without interspaces, the charge will be on the surface of the mass as a whole. A small charge will readily produce breaking up in the former case, but can only affect the most superficial particles in the latter. For further information the reader is referred to the papers of the investigators named.

Chick and Martin (1913) have also devoted a detailed investigation to the phenomena of "salting out," which should be consulted. We may note that the effect of hydrogen ion concentration shows that electrical charge plays a considerable part, as does also the effect of the valency of the precipitating ion.

From the preceding short account of the colloidal nature of proteins, it will be obvious that the phenomena presented by them are of much complexity, and are not yet altogether clear. Owing to their great variety in chemical constitution and the corresponding variety in their behaviour, it becomes almost a necessity to devote a special study to each one. There can be no doubt that their manifold capabilities of change in state make them very important in physiological processes. The effect of electrolytes, and especially of $H^{\cdot}$ and $OH^{\prime}$ ions, on this state may be emphasised. Adsorption of salts, especially of those which are comparatively insoluble, is also to be remembered. Since the degree of adsorption is proportional to the surface, it will be seen how, by alterations of state of aggregation, electric charge, or surface tension,

adsorbed, and therefore inactive, substances may be set free to manifest their activity, or "*mobilised*," to use a frequent form of expression.

The proteins of blood plasma do not appear to serve as food to the tissue cells. Quagliariello (1912, p. 174) showed that, when injected into the blood vessels, they are only utilised with extreme slowness. It appears that their chief value is due to their properties as colloids.

COMPLEX COLLOIDAL SYSTEMS

When a solution of an electro-positive colloid, such as ferric hydroxide, is added to one of an electro-negative colloid, such as arsenious sulphide, if the proportion of the two is such that the charges will mutually annul each other, both colloids are precipitated as a complex, and the solution is left free from both. This phenomenon has been investigated especially by Biltz (1904). The precipitate will, in such a case, have no charge. If excess of either colloid is present, only partial precipitation will occur, and both colloids will be present in the precipitate and in the liquid above, although in different proportion in the two. In other words, we have an adsorption compound formed, whose composition depends on the relative concentration of its components in the solution, and whose electric charge has the sign of that colloid which is in excess. The fact that only partial precipitation or mere aggregation takes place when either colloid is in excess is sometimes put in the form that the precipitate is soluble in excess of either colloid.

In such a comparatively simple system we see already conditions of much complexity, and when, in addition, emulsoid colloids, or proteins, are present, the possibilities are still more manifold.

The triple adsorption compounds of Rachlmann (1906) have already been described (page 65), and one or two examples of other complex systems may be instructive.

The fact that filter papers take up a greatly increased amount of Congo-red when its negative charge is reduced or reversed by cations, such as $Ca^{\cdot\cdot}$, has been previously referred to. Now, if gelatine be added, this effect is practically abolished, because the gelatine coats the paper with an emulsoid, itself insensitive to $Ca^{\cdot\cdot}$ ions. Egg-white behaves in the same way as gelatine, if in neutral solution ; but, if made acid (*i.e.*, electro-positive), it *increases* the action of $Ca^{\cdot\cdot}$ ions, and if alkaline, it *diminishes* their action, as in neutral solution (see Bayliss, 1906, p. 201).

The following experiment of Larguier des Bancels (1908, p. 198) is of interest in showing how an effect varies according to concentration: 2 c.c. of a dilute (0·125 per cent.) solution of aniline blue is totally precipitated by 5 drops of a certain ferric hydroxide preparation. If 5 drops of saturated ammonium sulphate be added in addition, only partial precipitation occurs; the solution is left deep blue. But if 40 drops of the ammonium sulphate be added, the precipitate is again nearly total.

As cases where we have to deal with complex mixtures of interacting colloids, we may mention: the coagulation of the blood, and the innumerable phenomena connected with hæmolysins, immunity, and anaphylaxis, together with intracellular processes in general.

An elaborate system of names has been introduced, especially in connection with hæmolysins and the coagulation of the blood, names which imply definite chemical individuals. From the complexity of the results to be obtained in colloidal reactions, from a very few distinct chemical substances, it seems more than probable that, as soon as sufficient knowledge is obtained of the nature of the phenomena in the systems referred to, the necessity for most of these names will be found to have disappeared. At present we find an investigator content to refer an experimental result to, say, "deviation of complement," apparently unaware that he is merely translating into a classical language what he has previously described in his mother-tongue. This particular case appears to be merely one of adsorption, a general phenomenon explicable on such fundamental laws as the principle of Clausius and Carnot. This question of terminology will be of necessity mentioned again (pages 307 and 328 below).

The papers of Gengou (1908) will be found very instructive in connection with the subject of the present section.

MODES OF PREPARATION OF COLLOIDAL SOLUTIONS

Certain processes have incidentally been given in the previous pages.

As a general statement, it may be said that the object to be attained is the formation of excessively minute particles of the substance which it is desired to obtain in the colloidal state. In the case of the suspensoid or irreversible class, it will be plain that we cannot take a portion of the dry solid and dissolve it in water in the way usually done in preparing solutions. This can be done with emulsoids in most cases, especially with the proteins; so that, if a preparation of colloidal silver, for example, is desired in the dry state, such that it can be made into a solution by mere addition of water, the method adopted is to coat the particles with some protein. This is done by adding such a protein to a solution of the suspensoid and then evaporating to dryness. The commercial "collargol" is such a preparation of colloidal silver.

In the case of the suspensoids, in order to prepare their solutions, the particles themselves must, as a rule, be formed in the liquid which is to be the medium of dispersion. Arsenious sulphide is formed by passing hydrogen sulphide through a solution of arsenious acid and sols of various metals by disintegration with the electric arc (Bredig) or spark (Svedberg) in the water or other liquid. In order that such sols shall be permanent, foreign electrolytes must be removed as far as possible.

It is indeed sometimes a difficulty in analytical work that precipitates will not deposit because of the absence of electrolytes to cause their aggregation. Sometimes it is possible to add a trace of an appropriate positive or negative trivalent ion, which will produce immediate clearing.

In traces, certain metals such as lead and copper pass into what seem to be colloidal hydroxides by mere contact with water, conferring certain toxic properties on the water. This is known as "oligodynamic" action, of which more will be said later.

Metallic hydrosols can be frequently prepared by reduction of their salts with various reagents, such as phosphorus or formaldehyde in the case of gold.

When a metallic salt is hydrolytically dissociated in water, prolonged dialysis removes the free acid, leaving the colloidal hydroxide. Instances are ferric and thorium hydroxides. In such cases, as also in those where the colloid is formed by double decomposition, an adsorption compound with the salt or precipitant is usually formed. For example, ferric chloride on dialysis gives a series of colloids containing less and less chlorine in relation to iron, from 3 of Cl to 1 of iron, to 1 of Cl to 400 or 500 of Fe, in no stoichiometrical proportion. If dialysis be continued until nearly all the chlorine is removed, the colloid tends to deposit rapidly; it seems to be stable only when in adsorption combination with a certain amount of the chloride.

To prepare emulsoids free from salts, as is frequently required, the only way is prolonged dialysis. Owing to the peculiarity of adsorption being relatively greater the lower the concentration of the solution of the adsorbed substance, it is a matter of much difficulty to remove the last traces of salts (Bayliss 1906, p. 181).

For colloidal dyes, Harrison's method (p. 71 above) is a useful means of purification.

In the production of a colloidal solution from a true solution, it will be clear that there is an increase of surface energy at the expense of a loss of osmotic energy (see p. 158). In such cases, the balance of energy changes cannot be stated without investigation of each case. If the colloidal solution is prepared by disintegration of a solid mass, as in Bredig's electrical method, the surface energy undergoes considerable increase, as does also the electrical energy, and, to a slight extent, the osmotic energy; so that energy must be supplied to the system by the electrical current. There is no simultaneous disappearance of the osmotic energy of a dissolved salt.

SUMMARY

Matter in the colloidal state is in the form of ultra-microscopic particles of solid, or droplets of fluid, in suspension or in other manner of dispersion, in another solid, liquid, or gas. A colloidal solution is a heterogeneous system, in the sense that

there are boundary surfaces of contact between the phases, although these phases cannot be readily separated by mechanical means.

Most of the characteristic properties of this state depend on the enormous development of surface in proportion to the total mass.

The chief factor in the stability of such systems, except those of two solid phases, is the Brownian movement of the particles; this movement is essentially identical with the molecular movement of the medium in which the particles are suspended.

There is reason to believe that, by appropriate means, any substance could be obtained in the colloidal state, and that substances usually met with in the colloidal state might be made crystalline.

The two great classes of colloids, emulsoid or lyophile, and suspensoid, or lyophobe, which are of the most importance in physiology, differ in the state of the internal phase, which is liquid in the former, solid in the latter. Other properties go along with these, and the names lyophile and lyophobe call attention to the relation of the dispersed phase to the liquid surrounding it. The internal phase in emulsoids frequently consists of a solid substance associated with varying amounts of the solvent, a fact which confers on it the properties of a liquid to a greater or less degree. The relative proportion of water, etc., in the two phases can be changed reversibly by various agencies, especially electrolytes.

The existence of finite particles in many cases can be demonstrated by the ultra-microscope, in which diffraction discs of light, sent off by the illuminated surfaces of the particles, are observed.

Owing to the dimensions of these particles, they are unable to pass through a membrane of colloidal substance, such as parchment paper or collodion; whereas crystalloids pass rapidly through these. The process is known as *dialysis* and is of frequent use to separate colloids from crystalloids. By the application of pressure, which must be greater than the osmotic pressure of the solution concerned, water also can be forced through, so that this process, known as "ultra-filtration," can be used to concentrate colloidal solutions.

When the internal phase consists of a substance capable of electrolytic dissociation in water, one ion being freely soluble and diffusible, it is found that the surface of the particles is dissociated in this way; the soluble ions move off as far as electrostatic forces permit, leaving the opposite ions concentrated on the surface of the particle, and giving it their combined electric charges. The giant multivalent ion so formed is called by Hardy a colloidal ion.

The possibility of a source of electrification akin to frictional electricity cannot as yet be definitely excluded as another source of the electric charge, usually found on the contact surface between phases.

The possession of this electric charge renders colloidal particles sensitive to the presence of ions of opposite charge. These neutralise the charges on the particles and cause precipitation, themselves being carried down with the precipitate. In this process, the effect of valency is out of all proportion to the increased number of charges.

In the case of emulsoids, which are less sensitive than suspensoids to this purely electrical effect, neutral salts have a further action, shown in its most marked form as "salting out"; but in lower concentration than necessary for this purpose, they have an action due to their effect on the general properties of water, altering its distribution in the two phases of the system, and therewith other properties, such as surface tension, viscosity, compressibility, coagulation time, etc. This phenomenon may be brought into relation with the association of part of the water with the ions of the electrolytes.

There is also evidence of adsorption of salts in the case of proteins; but whether any true chemical combination occurs is doubtful.

Certain emulsoids, such as gelatine, have the property of forming semi-solid

structures known as *gels*. This has been shown in some cases to depend on a redistribution of phases, so that the more solid one changes from the position of internal or dispersed phase to that of external or continuous phase.

Another important character of emulsoids is that of *imbibition*, by which they take up large amounts of water, swelling in the process, and exercising considerable force. Acids and alkalies increase the amount of water taken up in the process. The effect of neutral salts in the main follows the same law as the precipitating action, but it seems necessary to assume an additional factor, probably adsorption.

In imbibition, there are probably two processes at work, one the condensation of water on the surfaces of the colloidal elements, the other, solution of the water in the substance of the particles. No doubt the relative part played by each varies with the amount of water at the disposal of the colloid.

Imbibition is incapable of explaining the changes of volume of living cells under the action of crystalloids.

Proteins are emulsoids and obey the same laws as other members of the class. As amphoteric substances, they form salts with strong acids or bases, which salts are electrolytically dissociated. In the first case the protein ion, colloidal, is the positive one, so that the particles forming the internal phase will possess positive charges; in the second case, it will be the negative one.

Since the acidic and basic groups may not be of exactly equal strength, proteins are sometimes naturally electrically charged by surface ionisation of the kind described above.

The effects of acid and alkali on the physical properties may be accounted for by the properties of the protein ion, formed in various relative amounts in different cases.

Certain proteins are capable of a change, known as "denaturation," in which their properties approximate to those of a suspensoid, especially in regard to their sensitiveness to electrolytes, in accordance with Hardy's rule of valency.

The phenomena of aggregation and mutual action, presented by mixtures of colloids and crystalloids, offer great complexity and are of much importance in physiological problems, although as yet very inadequately worked out.

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CHAPTER V

THE PERMEABILITY OF MEMBRANES AND THE PROPERTIES OF THE SURFACE OF CELLS

An amœba, after having taken in a vegetable cell, proceeds to digest the substances contained therein. The products, in order to serve as food, must diffuse from the digestive vacuole into the other parts of the protoplasm. But, if they were able to diffuse out from this protoplasm into the water around, they would be lost to the organism. There is good reason to believe, therefore, that there must be some layer or film on the outer surface of an amœba through which dissolved non-colloidal substances, such as sugar and amino-acids, cannot pass.

Evidence was given in our first chapter to show that living protoplasm must have the properties of a liquid. This fact also points to the necessity of some kind of an envelope, otherwise the organism would stand great risk of colloidal dispersion through the water.

The nature of this limiting membrane, with respect to the substances which it allows to pass through, and those which are kept back, is of much importance.

THE PROPERTIES OF MEMBRANES IN GENERAL

It is obvious that a membrane, being merely a thin sheet or film, may be composed of almost any substance. But, for our purpose, it is useful to classify membranes according to their behaviour towards water, and towards substances dissolved in it. In the first place, there are such things as glass or mica, which allow neither water nor substances dissolved in it to pass through. Such may be called *impermeable* and have a comparatively small importance. There are also some materials which are impermeable to water, but allow certain other liquids or gases to pass through; for example, india-rubber is impermeable to water, but allows pyridine to pass through. A metal, palladium, may be regarded as impermeable to water under ordinary circumstances, but allows hydrogen to pass through. Such cases are of interest in certain problems.

The most important membranes for the physiologist are those which allow water to pass through, but hold back dissolved substances. There are various degrees in this respect; some membranes, such as parchment paper, gelatine, etc., will not allow colloids to pass, but are freely permeable for crystalloids. Copper ferrocyanide, on the other hand, holds back the majority of both colloids and crystalloids, but allows water to pass. A membrane which does not permit any dissolved substance to pass, while permeable to water, is known as *semi-permeable*. Such a membrane has not been prepared in the laboratory, although the copper ferrocyanide of Traube approximates to it very closely. When we wish to speak of a membrane which allows water to pass, but not a particular given substance, we say that it is semi-permeable as regards that substance.

Membranes may also be looked at from another point of view, that of their *structure*. This may be of the nature of a sieve, so that different membranes have different sizes of holes. Or a membrane may allow certain substances to pass through it because of their solubility in the substance of which the membrane is composed. Or, thirdly, they may possibly form reversible chemical compounds with the substance to which they are permeable. The two last cases need not delay us long at this stage. As a case of a membrane which is permeable by a substance, because of the solubility of this substance in the membrane, we may take

a membrane of water, supported in some way, as in wet parchment paper. This allows carbon dioxide to pass through, but keeps back oxygen and nitrogen. Consideration will show, however, that, since these latter gases are not absolutely insoluble in water, after a sufficiently long time there will be no difference in composition between the gaseous mixture on the two sides of the membrane. A membrane of palladium, as investigated by Ramsay (1894), is permeable to hydrogen, but not to oxygen, either because the hydrogen is soluble in it, or because a reversible compound of some kind is formed, which dissociates under a lower tension of hydrogen.

M. Traube

FIG. 44. PORTRAIT OF MORITZ TRAUBE.

As regards membranes like parchment paper, gelatine, collodion, etc., which allow water and crystalloids to pass, but hold back colloids, it is practically certain that they have a porous structure. Many facts point to this. Biltz (1910) showed that the rate of passage of dyes through parchment paper is in direct relation to their molecular dimensions. Heymans (1912) found that certain micro-organisms were able to pass through this paper. When speaking of the ultra-filter of Bechhold, I stated that the permeability could be varied by taking different strengths of collodion, and Bechhold himself (1908) has determined the dimensions of the pores of various membranes by pressing air through them, when covered with water. Schoep also (1911) has been able to control the dimensions of the pores by mixing castor oil and glycerol with the collodion used to prepare the membranes.

The copper ferrocyanide membrane has played a great part in the investigation of osmotic pressure; the discoverer of it will be of sufficient interest to the reader to warrant the introduction of his portrait (Fig. 44).

When a solution of potassium ferrocyanide comes into contact with one of copper sulphate, a membrane in the form of a colloidal gel of copper ferrocyanide is formed at the surface of contact. This gel contains a considerable percentage of water; if allowed to dry, it becomes impermeable altogether, even to water. In order to be able to perform experiments with such a membrane, it must be supported by being formed in the pores of a cylinder of unglazed porcelain, or, in some cases, in collodion. Further details will be found in the chapter on osmotic pressure. This membrane, although colloidal, obviously has interspaces between its constituent elements of much smaller dimensions than those of gelatine or collodion, since, as Traube showed (1867), it does not allow cane-sugar to pass through, nor even many salts. Its discoverer regarded it as a "molecular sieve," in that its pores, while large enough to allow water to pass through, were too small to admit dissolved substances. Closer investigation, however, showed that there are some salts which can pass through. Thus, potassium chloride was found to do so, while barium chloride, calcium chloride, potassium sulphate, barium nitrate, and ammonium sulphate could not.

It is pointed out by Ostwald (1890) that it is not necessary to assume that a membrane is impermeable to *both ions* of a salt, when it is found that the salt in question is not allowed to pass. If one ion only is allowed passage

electrostatic attraction on the part of the oppositely charged ions will prevent the permeable ion from travelling further than such a distance at which its osmotic pressure balances the electrostatic force. Copper ferrocyanide is permeable to both ions of potassium chloride; therefore, when it is found to be impermeable to calcium chloride, it must be the calcium ion which is held back. Similarly, in the case of potassium sulphate, it must be the SO_4'' ion to which the membrane is impermeable.

The fact that the membrane is not completely semi-permeable has led some observers to hold that its permeability or otherwise is a matter of solubility in the substance of the membrane itself. This view does not really lead us any further, and, if we introduce the modern conception of the hydration of solutes, and especially of their ions, it is still possible to look upon the membrane as a sieve. Substances when dissolved become associated with a number of molecules of the solvent, varying with the chemical nature of the solute. Thus, according to J. C. Philip (1907), each molecule of potassium chloride has 7 to 11 molecules of water associated with it, while copper chloride has about 21, and so on. Another fact, which tends to support Traube's view, is that, as he found, a copper ferrocyanide membrane, permeable to potassium chloride, becomes impermeable to it when infiltrated with silver chloride (Traube, 1899, p. 261). It does not seem likely that there should be any material difference between the solubility of potassium chloride in silver chloride or in copper ferrocyanide; if any, one would expect it to be more soluble in the chloride, according to the old law, "similia similibus solvuntur" (Rothmund, 1907, p. 112). On the other hand, it is to be presumed that any pores present would be narrowed by deposition of silver chloride on their walls.

A detailed investigation of the permeability of a large number of precipitation membranes was undertaken by Paul Walden (1892). If the table on pp. 716 and 717 of his paper be consulted, various facts will be noted which have a bearing on the question before us. The membranes can be arranged in order of merit, as regards impermeability to the substances tested. Tannin-gelatine is the lowest in the series, being permeable to all except tannin itself; while copper ferrocyanide is the highest, being impermeable to a larger number than any of the others. A significant fact is that none of the membranes comes out of its place as regards any particular substance. That is, assuming that the pores increase regularly in dimensions from the copper ferrocyanide to the tannin-gelatine, no substance is found which diffuses through a membrane having the smaller pores while being held back by that with the larger pores, as might happen on the solution theory. The behaviour of the hydrochlorides of the three ethylamines is of interest. The copper ferrocyanide membrane is readily permeable to that of monoethylamine, slightly permeable to that of the diethylamine, impermeable to that of the triethylamine, following the increase of molecular dimensions.

The difficulty frequently arises, however, as to the proof that the membrane is not chemically acted upon, or injured in its integrity, when it appears to be permeable to a particular solute. This consideration seems to deprive Tammann's experiments with dyes (1892, p. 257) of much of their value, although this observer draws the conclusion that there are dyes which pass a membrane which is supposed to have the smaller pores, while being held back by one with the larger pores, and that Traube's theory does not hold. In Walden's experiments, the permeability of the membranes composed of the ferrocyanides of zinc and of copper is identical, whereas in those of Tammann the zinc membrane shows itself to be permeable to dyes to which the copper one is not; it is even stated to be permeable to "Baumwollenblau" to which the tannin-gelatine membrane is impermeable, and even parchment paper only slightly so. If we neglect quantitative differences, which are very difficult to judge satisfactorily, there are only two out of Tammann's seventeen dyes which fall out of line. "Baumwollenblau" is one of these and the other is fuchsin-chloride, to which copper ferrocyanide is permeable, zinc ferrocyanide not. According to Cain and Thorpe ("Synthetic Dye-stuffs," 1913) "cotton-blue" is a mixture of ammonium and sodium salts of di- and tri-sulphonic acids of rosaniline blue. Since even parchment paper is impermeable to the salt of the mono-sulphonic acid ("aniline-blue") it is difficult to believe that a zinc ferrocyanide membrane (if perfect) should be permeable to the "cotton-blue" mixture. The experiments of Biltz (1910, p. 117) on the passage of dyes through parchment paper, have been referred to above. These experiments show an unmistakable relation between the molecular dimensions of the dye and its ability to pass through the paper. If the number of atoms is less than 45, it passes through quickly; above 45, slowing begins to show itself; between 55 and 70, the passage is very slow; and about 70, it ceases altogether. Of course, the actual space occupied

by a molecule does not depend only on the number of atoms it contains. The chemical arrangement must also be taken into account; accordingly, chemical structure was found to have some effect on the results. The "sieve theory," then, appears to hold in the case of colloids and, as we cannot draw a line of demarcation between them and crystalloids, the general application of the theory receives support.

Abel (1914) finds in his "vividiffusion" method, that the rate of diffusion through collodion membranes is independent of their thickness, a fact which suggests pores rather than solution in the substance of the membrane.

When we recollect that the copper ferrocyanide membrane is freely permeable to water, in fact, contains water in its constitution, it seems not so easy to understand how a substance such as sugar, which is easily soluble in the water contained in the membrane, fails to pass through, unless something like a sieve is present, opposing a mechanical constraint on molecules above a certain size.

Tinker's photographs (1916, 1917) show that actual pores are present in copper ferrocyanide membranes, although the actual measurements are uncertain, owing to diffraction. This author holds that the pores are narrowed by adsorption of solvent, and that osmosis is caused by difference between the "hydration" of the two sides of the membrane. Meigs (1915) concludes that membranes of identical chemical composition differ according to their physical state.

Bartell (1911) showed that, when water was forced by pressure through a membrane of copper ferrocyanide, the rate at which it flowed through obeyed Poiseuille's formula for the case of capillary tubes.

But, before the question at issue can be finally decided, it will be necessary to understand more completely the nature of the process of solution, and it may very probably be found that there is no real contradiction between the two opposing views.

With regard to the structure of colloid membranes in general, it will be clear that the remarks on page 14 above are of importance. If a membrane of gelatine has a honeycomb structure, any substance passing through it must traverse a structure consisting of much finer pores than if the membrane were of a sponge-like nature, where it could pass, by a tortuous channel, between the actual trabeculæ of the solid phase.

Another point to be remembered is that the surfaces of the elements of the membrane adsorb dissolved substances. In the filtration of salts through a gelatine filter, the first portions of the filtrate contain less salt than the original solution; this continues until the adsorption capacity of the membrane is saturated. A colloid, when adsorbed, may diminish considerably the dimensions of the pores, so that the filter becomes impermeable for substances to which it was at first permeable.

It is frequently found that a solute, to which a membrane appears to be impermeable, will pass through in very small amount, if allowed a long time. There are two possible causes for this fact. The pores in an artificial membrane are not all of exactly the same size, as was noticed by Bechhold in his measurements of various membranes. Suppose that there are a few of them which will allow a certain solute to pass, while the great majority are impermeable to it; it will take a long time for an appreciable amount of the solute to find the small number of channels available for it, owing to the slowness of diffusion. A similar state of affairs would be found if the particles of the solute varied in dimensions, even if the membrane were of a uniform structure.

These facts lead to reference to the rate of passage through a membrane. In addition to the factors mentioned in the previous paragraph, a little consideration will show that a membrane may be freely permeable to a solute, but, if the rate of diffusion is very slow, comparatively little will pass in unit time, owing to the supply at the surface of the membrane not being kept up sufficiently. This state of affairs plays a part in certain osmotic phenomena to be discussed in the next chapter.

THE SURFACE MEMBRANE OF THE CELL

The present chapter was commenced by pointing out the necessity for some arrangement by which, in such organisms as the amœba, sugar and other soluble food-stuffs are prevented from diffusing out and being lost to the protoplasm.

From what we have learnt in the preceding section, it is plain that what is needed is a membrane with properties similar to those of Traube's copper ferrocyanide, but more perfectly semi-permeable.

As we shall learn in more detail later, there is a remarkable similarity between the properties of the cell membrane and those of the artificial one, although it is not to be supposed that they have anything in common as regards their chemical nature. Both are permeable to ammonium chloride, impermeable to ammonium sulphate. It is usually stated that the cell membrane is impermeable to potassium chloride, while the copper ferrocyanide membrane, as we have seen, is freely permeable to it. But this statement needs qualification. Overton (1904, pp. 188-209) has shown that the muscle cell is not completely impermeable to potassium chloride and that, in fact, potassium salts fall into two groups, the first, typified by the sulphate and phosphate, to which complete semi-permeability exists, and the second, typified by the chloride. It will be noted that this behaviour is similar to that of the copper ferrocyanide membrane, which, according to Walden (1892), is permeable to chlorides, bromides, iodides, and thiocyanates, impermeable to sulphates, phosphates, and oxalates. The muscle cell, however, is only very slowly permeable by potassium chloride. Meigs (1913), moreover, finds that a celloidin membrane, impregnated with calcium phosphate, has most of the properties of the cell membrane, as regards permeability. It is impermeable to the chlorides of sodium, potassium, and calcium, to cane-sugar and alanine, somewhat permeable to glycerol and urea, freely permeable to alcohol. Although it seems scarcely likely that the cell membrane is actually composed of calcium phosphate, it is important that an artificial membrane of nearly perfect semi-permeability can be prepared. Philippson (1913), again, shows that, if collodion membranes are impregnated with an ethereal extract of muscle, they become almost impermeable to inorganic acids, while retaining their permeability to organic acids, increasing in the series, formic-acetic-lactic-butyric. This result is of interest as a further step in the artificial production of membranes with properties similar to those of the cell membrane.

That a membrane of some kind is actually formed on the surface of contact between protoplasm and water is shown by the observations of Kühne and of Pfeffer referred to below (page 128). If any substances are present in the cell which lower surface energy, we know that they will be concentrated at the surface, and from Ramsden's experiments (page 55) we are prepared to find that a coherent membrane will probably be formed. It is not necessary, then, that an actual visible skin should be present, although in certain cases it appears to exist. Moreover, the kind of membrane contemplated in the statement just made forms, or may be regarded as, an integral part of the living protoplasm itself, and as long as this is living, will probably share its power of change and adaptation in response to changes in the environment. This point of view will require further treatment later (see also Chambers, 1917).

When we come to the constituent cells of higher organisms, which are dependent for their food supply on substances in the blood or other liquid bathing them, we are at once met with a difficulty, if we assume the existence of such a semi-permeable membrane. If it prevents food-stuffs from being washed out of the cell, it must also prevent them from getting in.

This difficulty has caused certain investigators to deny altogether the existence of a membrane impermeable to electrolytes and other crystalloids. Martin Fischer and Gertrude Moore (1907, p. 342), for example, appear to hold that imbibition by colloids is capable of explaining the phenomena for which a semi-permeable membrane was postulated.

In order to understand the nature of the evidence on this question, it is necessary to forestall somewhat a part of the subject matter properly belonging to the chapter on osmotic pressure. Suppose that we have a vesicle, say of copper ferrocyanide, containing a solution of sugar, and that we immerse it in water. Since the membrane is impermeable to sugar, but permeable to water, the sugar molecules inside exert a pull on water molecules which enter and distend the

vesicle, by the process known as osmosis. This must for the present be taken as an experimental fact. If the water outside be replaced by a solution of sugar, but of a lower concentration than that within the membrane, water will enter until the concentration is equal on both sides; if the solution outside is stronger than that inside, water will escape, until again the concentration is the same on both sides. It is not a necessity, moreover, that the two solutions, inside and outside, be of the same substance, so long as the membrane is impermeable to it. The amount of distension or collapse is clearly in exact proportion to the molecular concentration of the solutions, since on this depends the degree of dilution or concentration necessary to bring the inner and outer solutions into osmotic equilibrium. Now, careful investigations of the behaviour of the cells of the kidney by Siebeck (1912) and of the muscle cells by Beutner (1912, 2, and 1913, 3) have shown that living cells react in the same way as the semi-permeable membrane described above. The changes in volume are simply proportional to the molar concentration of the solutions used.

All the various members of the "Hofmeister series," in equal concentration, have the same effect. The process of imbibition, as we have seen (page 100 above), follows a different law. The series of electrolytes just referred to, in equal concentration, have different effects on imbibition according to their action on the properties of water, so causing it to be distributed between the two phases of the colloidal system in a different proportion. Moreover, sugar behaves, as regards its effect on the volume of cells, just as a salt of the same osmotic pressure, provided that the salt is one to which the membrane is impermeable, whereas, according to certain investigations, it is devoid of action on imbibition processes. Martin Fischer and G. Moore (1907, p. 339) find that non-electrolytes in general have no effect on the swelling of fibrin.

Further facts are, I think, unnecessary to show that the imbibition theory is insufficient to account for more than a small part of the behaviour of cells towards solutions of varying concentration. At the same time, there is no doubt that the power of changing the water content of cell constituents must play an important part in cell mechanics.

We may now pass on to consider the nature and properties of the cell membrane. It will clear the way somewhat if I state the general conclusion which is forced upon us by consideration of the whole of the evidence on this disputed question, although, at first sight, it may seem rather a lame one. It is, in fact, that the cell membrane is *sometimes permeable* to crystalloids, *sometimes not*. This will seem more satisfactory when we find that the apparently capricious behaviour is in relation to functional changes in the cell, or dependent on the action of definite substances. As regards colloids, the membrane itself is probably always impermeable; although in special cases, as the cells of secreting glands, there appear to be arrangements by which colloids can get in or out, probably by rupture of the membrane.

Impermeability to Crystalloids.—If a slice of living red beetroot be allowed to soak in tap water, it will be found that neither the red pigment nor the cane-sugar escapes from the cells. This fact can only be explained on two hypotheses: either the cell membrane is impermeable to these substances, or they are combined in an irreversible manner with the insoluble matter of the cells. Now, Moore and Roaf (1908, p. 80) appear to regard the existence of some kind of chemical combination between the proteins of cell protoplasm and electrolytes as sufficient to account for the difference of composition between cell and surrounding liquid, without the necessity of assuming the existence of a semi-permeable membrane. But, if this compound is reversible, as an adsorption process would be, there can be merely a *quantitative* difference between the cell contents and the outer solution, because an adsorption process is only in equilibrium with a finite concentration of adsorbed substance in the solution with which the surface is in contact. This is contradictory to experience in the case of the beetroot, and we shall find other instances as we proceed. If the hypothetical compound is a more strictly chemical one, it must be neither hydrolytically nor electrolytically dissociated, and, in fact, completely insoluble and inert. It is difficult to see of what value such a substance can be in the dynamics of the cell. Moreover, direct measurements by Höber (1912, 2) of the electrical conductivity of the interior of cells show that a part, at least, of the inorganic constituents are *free*.

We are compelled, therefore, to assume the existence of a membrane of some

kind, and the question to be answered is: Must the membrane be of necessity impermeable to electrolytes and other crystalloids, or is it sufficient if it is impermeable to colloids? It is plain that if the latter alternative is found to be satisfactory, less difficulty will be found in imagining an adequate structure. It will be remembered that no artificial membrane is known as yet semi-permeable as regards potassium chloride, for example, to which the cell is usually semi-permeable, but important steps have been taken already in this direction as mentioned above (page 115).

The chief evidence may be grouped conveniently under three heads: (1) The phenomena of changes in volume and internal pressure under the action of solutions of various concentrations. (2) The difference between the cell and the surrounding medium as regards presence and concentration of crystalloids. (3) The resistance of living cells to the passage of electrical currents through them.

1. When cells or blood corpuscles are placed in solutions of crystalloids of various concentration, it is found that in the case of most of these, provided that they do not injure the cell, there is a particular concentration in which no change of volume of the cell occurs. With solutions of a greater strength than this, a shrinking takes place, and with weaker solutions, a swelling. On the theory that these results are of osmotic origin, the solution which causes no change is called "*isotonic*," and the others "hyper- and hypo-tonic" respectively. But the matter is not quite so simple as it might appear at first. The word "isotonic" implies that the solution which causes no change in the volume of the cells has the same osmotic pressure as the normal contents of the cell. How far this is true depends on the permeability of the membrane, as the following considerations will show. Suppose that we have a 5 per cent. solution of sugar enclosed in a bag of an elastic membrane, which is permeable to water, but impermeable to sugar, and that this is immersed in water. Water will enter the bag, which will be distended and probably ruptured, unless supported by an outer envelope, such as the cellulose wall of plant cells. The pressure developed when the cell is not allowed to increase in volume is the full osmotic pressure of the sugar solution. The tense condition of the cell hereby produced is known as "*turgor*," and is the normal state of the plant cell, enabling the stems of the higher plants to remain rigid and erect, as long as the cell membranes retain their semi-permeable properties. That very considerable pressures do exist within plant cells is obvious from consideration of the growing cambium layer between the wood and the bark of a tree. Growth takes place at this situation, so that the wood is continually being increased in diameter; it is clear, therefore, that the bark must have an enormous stretching force being continually applied to it, and that the growing cells must be exposed to great pressure, which would crush and kill them unless opposed by an equally great pressure within them. The stretched state of the bark can be seen by removing a ring of it, after cutting it through at one place. If it be then replaced in position, it will be found that the ends cannot be made to meet. Fig. 45 represents this fact. From the tension required to stretch the bark to its original length, the pressure exerted on the cambium cells can be calculated. It is common to find in plant cells pressures as high as 15 atmospheres. Now, pressures of this order can only be maintained either by osmotic forces or by imbibition. The construction of a plant cell, with its inner cavity of solution surrounded by a protoplasmic membrane, suggests at once an osmotic machine,

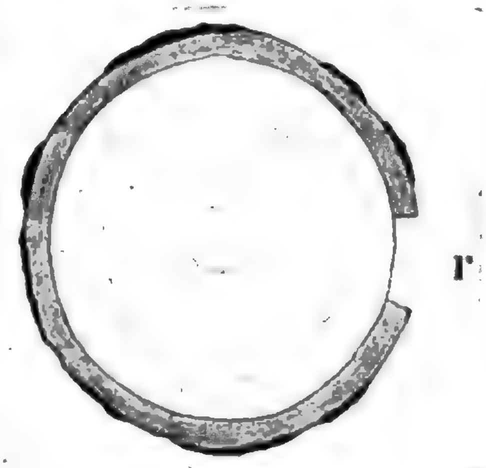

FIG. 45. TO SHOW THE PRESSURE EXERTED ON GROWING CAMBIUM CELLS.—Cross section, slightly enlarged, of an internode of a Holly branch, from which the bark has first been removed and then replaced around the woody core. A very great tension is required to make the ends meet again at *r*.

and we have already seen that imbibition is incapable of explaining the phenomena met with. From the molecular concentration of the cell sap, as determined by the depression of the freezing point, in the way explained in the next chapter, or in other ways, the maximum possible pressure that could be developed if the membrane were completely semi-permeable can be known. Although it is naturally a matter of difficulty to obtain the juice of one kind of cell alone, it appears from results obtained that, on the whole, the concentration of the cell sap is not greater than is necessary to give the turgor pressure known to exist.

A large number of measurements of depressions of freezing point will be found collected in the article by Bottazzi (1908); the usual figures correspond to pressures of about 11·15 atmospheres and would be given by a solution of potassium nitrate of nearly half molar strength (5·05 per cent.).

The shrinking of a cell placed in hypertonic solutions shows itself in plant cells by the protoplasmic layer retreating from the rigid cell wall, leaving a gap between the two. This phenomenon is known as "*plasmolysis*," which was worked out mainly by de Vries (1884), and has played a large part in the investigation of the permeability of cells.

To interpret the facts observed when cells are exposed to solutions differing in osmotic pressure from that of the cell contents, let us return to the schema of the cell, viz., a solution of some substance contained within a membrane forming a vesicle, which can be immersed in water or solutions of various osmotic pressures. Suppose, first, that the membrane is impermeable to the solute, and that the vesicle is immersed in a slightly hypotonic solution of the same substance. The vesicle will at first absorb water, becoming distended, until its contents are diluted to such a degree that their concentration is equal to that of the outer solution. Nothing further will happen, but the cell remains permanently distended.

Next, let us imagine that the membrane is easily permeable both to water and to the solute, and that it is elastic as before. It is clear that, in this case also, the cell will be distended to begin with, because the osmotic pressure is greater inside than outside, while the solute cannot escape instantaneously. But subsequently, and contrary to the previous case, the original volume will be regained. As the solute gradually escapes, the internal osmotic pressure becomes equal to the external by free diffusion, and there can be no permanent force to keep the membrane stretched. In the previous case, the cell could return to its original volume only by escape of water; but, since the solute could not escape, the original concentration would by this means be arrived at and equilibrium would no longer exist. Now, there may be numerous degrees of permeability between the two cases given, such that the solute may be able to escape at different rates. The result is that a longer or shorter time would elapse before the cell returned to its original size. In both cases, however, if no change of volume occurs at all, the conclusion may be drawn that the outer solution is isotonic with the contents. If the change of volume is only temporary, while the membrane is elastic, it is to be concluded that this membrane is more or less permeable to the solute.

Another case to be considered is one that is met with in certain experiments on living cells or blood corpuscles, viz., when the membrane is permeable for the solute of the outer liquid, but impermeable for those of the cell contents. Suppose that the two solutions are isotonic. No immediate change will take place. But, presently, the cell will begin to swell. Why? Because the solute of the outer solution passes into the cell, so that the osmotic pressure therein is now the original one plus that of the substance which has diffused in; while the outer solution remains the same as before, always assuming, as in all the cases discussed, that the volume of this solution is large compared with that of the cell. Ultimately, the state of affairs will be the same as if the outer liquid had been water only, since the concentration of the diffusible solute is equal on both sides of the membrane of the cell, while the latter retains the whole of the indiffusible substance with its osmotic pressure.

It appears that, unless we know that the cell membrane is elastic, some uncertainty may arise as to the conclusions to be drawn from the effect of a solution which is not isotonic

with the cell contents. Suppose this solution to be hypotonic. The cell will at first increase in volume, as we have seen, whether the membrane is permeable to the solute or not. If it is impermeable to the solute, this increase in volume is permanent. But, if the increase in volume is not permanent, the cell must be more or less permeable. On the other hand, it seems possible, if the membrane is inelastic, that a permanent increase in volume might result from a hypotonic solution, even if the membrane is permeable to the solute. The first effect having been to dilute the contents until their osmotic pressure is equal to that outside, while the membrane has allowed itself to be stretched without any elastic reaction, there does not seem to be any force capable of returning the cell to its original volume. This being so, caution is necessary in drawing conclusions, unless it is definitely known that the membrane is elastic.

Calculations made by Roaf (1912, i. p. 145) make it probable that equilibrium between diffusible substances inside and outside the cell takes place with great rapidity, so that it is possible that a process requiring seven days for equilibrium in an osmometer with parchment paper might be complete in 0·001 minute in the case of a cell, owing to the very large surface in proportion to volume in this latter case. It is justifiable to assume, then, that osmotic equilibrium of substances to which the membrane is permeable takes place practically almost instantaneously. But, at the same time, in the case of partial permeability, that is, if we regard the sieve as having only one hole in a thousand large enough to permit the passage of the molecules of a particular solute, the rate of diffusion of this solute through the membrane can be only about 0·001 of that of another solute, which can pass through *all* the pores.

Some experiments, made by Overton (1902) on the sartorius muscle of the frog, serve to show the impermeability of cells to crystalloids. When placed in 0·7 per cent. sodium chloride, there was no change in weight, even in several hours; hence this solution is isotonic with the muscle (Overton, p. 129). Suppose we add another substance to such a solution, if the muscle cells are impermeable to it they must shrink in order to increase their osmotic pressure by loss of water. Overton adds methyl alcohol to the extent of 5 per cent. No effect is produced; hence the cells are permeable to methyl alcohol (p. 167), for this concentration of methyl alcohol raises the osmotic pressure of the salt solution very considerably. If the substance added is slowly permeable, a mixture of effects results. A muscle placed in a solution containing 0·35 per cent. sodium chloride, and 3 per cent. ethylene glycol, *i.e.*, a solution whose osmotic pressure is equal to that of a 2 per cent. sodium chloride and therefore considerably hypertonic, loses weight at first, as if impermeable to glycol, but afterwards gains weight. The explanation is that the glycol can penetrate slowly, so that, after a time, its concentration within and without the cell becomes equal and the effect of 0·35 per cent. sodium chloride, which is hypotonic, remains alone (p. 195). As to the third possible case, glucose when added produces the same effect as sodium chloride of the same osmotic pressure, viz., permanent shrinking; hence the membrane is impermeable to it (p. 224).

There remains the possibility to be considered, whether the apparent impermeability to salts may not be sufficiently accounted for by the existence of a membrane semi-permeable as regards colloids only, but permeable to electrolytes, as appears to be the view taken by Roaf (1912, i. p. 145). Ostwald (1890) has pointed out that it is sufficient for a membrane to be impermeable to one ion only of an electrolytically dissociated salt in order that neither ion shall pass through. Suppose, therefore, that we have a salt of a protein present, which may be one with an acid to which the membrane is permeable, or a base of similar permeability. If this salt is not hydrolytically dissociated, the fact that the colloidal ion does not pass out will prevent the opposite diffusible ion from doing so. But in such a case the colloidal salt must be present without any colloidal salt of the other kind; that is, we cannot have two colloidal salts, in one of which the anion is diffusible and in the other the cation.

For example, if there were a hydrochloride of a protein, and the sodium salt of a protein together, the positive and negative inorganic ions would escape together, or sodium chloride would diffuse out, without let or hindrance from electrostatic attraction on the part of the colloidal ion.

The hypothesis of a membrane impermeable only to colloids will not, therefore

explain the semi-permeability of the cell to neutral salts. We have seen above that there is no satisfactory evidence of combination between proteins and such salts, and, moreover, the hypothesis in question leaves the impermeability to glucose unaccounted for. Glucose does not form a compound with proteins of the kind required, and according to Asher (1912) exists *free* in the blood.

A further difficulty lies in the high osmotic pressure in certain cells; to obtain a pressure of 11 atmospheres, a half molar solution is necessary, and when we remember that the molecular weight of proteins is about 2,000, we see the impossibility of such a solution. The total solid content of cells is only about 20 per cent., and of young, growing, cambium still less. Substances of small molecular weight only can give the observed osmotic pressure.

The hæmatocrite (Hedin, 1891), as applied to problems in permeability (Höber, 1910), is a practical use of the facts described in the preceding section.

2. We pass on to discuss some facts relating to the distribution of crystalloids between the cell and the surrounding medium, which necessitate the presence of a membrane impermeable to crystalloids. These facts are of interest in other ways.

The red blood corpuscles of the rabbit contain much more potassium than the plasma which bathes them, and no sodium at all, according to the analyses of Abderhalden (1898, p. 100). Thus:—

	Plasma.	Corpuscles.	
Potassium	0·259	5·229	per thousand.
Sodium	4·442	0	

Such relations are impossible to account for except on the assumption of a membrane impermeable to sodium and potassium, unless these substances are combined with the colloids in an irreversible, non-dissociable, manner. It is easy to show, moreover, that the salts of blood serum readily pass through a membrane of parchment paper, which is impermeable to colloids, since they are frequently removed in this way. If the membrane of the rabbit's blood corpuscles were impermeable only as regards colloids, sodium salts from the serum must inevitably pass through.

It is true that, under certain conditions, as was found by Donnan (1911) and by myself (1911, ii. p. 249), independently, there may be different concentrations of a freely-diffusible salt in equilibrium within and without a membrane of parchment paper. This fact is brought by Roaf (1912, i. p. 145) in support of the opinion that a membrane impermeable to electrolytes is unnecessary, so that it must be considered briefly. Take the case of the sodium salt of a protein or of Congo-red, in solution inside a membrane of parchment paper. As long as water only is present on the other side of the membrane, the sodium ions cannot escape further than the position in which their osmotic pressure is balanced by electrostatic attraction to the opposite, colloidal, ion inside. A Helmholtz double layer is formed, the sodium ions being outside. Now it is not to be supposed that the same individual ions are always present in this double layer; a perpetual interchange is going on between them and those present in the body of the solutions. Moreover, since their position is due solely to the fact of their possessing a positive charge, it is clear that if any other cations are in a position to interchange with them, the process will take place. This state of affairs will exist if any salt, say potassium chloride, is present in the outer solution. The external component of the double layer in such a case will consist of both $K^{\cdot}$ and $Na^{\cdot}$ ions in relative proportion, according to their respective concentrations in the solutions, and ultimately this same proportion will be established throughout both solutions, whatever the absolute concentration of the ions therein. This fact was pointed out by Ostwald (1890, p. 714) as applying to the copper ferrocyanide membrane and found experimentally by W. A. Osborne (1906) in the case of salts of caseinogen, or soaps within a parchment paper membrane, and by myself in that of Congo-red or of serum proteins in similar conditions. Although the ratio of the concentrations of the diffusible salts is the same on both sides of the membrane in such cases, as already remarked, the absolute concentration is greater on that side containing the colloidal solution. This fact seems to be due to the necessity that the concentration of *non-dissociated* salt must be equal on both sides; there are, in fact, so far as one can see, no forces present capable of making possible a different concentration of electrically-neutral, freely-diffusible, substances. If, then, we have say sodium chloride in decimolar solution on the outside, and the sodium salt of Congo-red inside, assuming 10 per cent. of the sodium chloride undissociated, this concentration of undissociated molecules must be the same inside; this cannot be the case if the total concentration of the chloride is the same on both sides, since that inside will be less dissociated than that outside, owing to the presence of the dye salt with an ion ($Na^{\cdot}$) common to both salts. This explanation of the unequal distribution of sodium chloride on the two sides of a membrane applies also if the diffusible salt placed outside has not, to begin with, an ion in common with the colloidal salt, say potassium chloride, because, as pointed out above, after equilibrium is attained, there will be present both inside and outside all the kinds of the diffusible ions of the system. This

I have shown experimentally to be the case, while Donnan (1911) has deduced it from thermodynamic considerations.

We see, therefore, that the presence of a colloidal salt within a membrane, semi-permeable only as regards colloids, will not account for the unequal ratio of potassium to sodium in the plasma and corpuscles of the rabbit.

Consider next the case of the muscle cell. The experiments of Katz (1896, p. 42) have shown that, in the rabbit, the ratio of the sodium to the potassium in these cells is as 0·46 to 4; whereas, as we have seen, the corresponding numbers for the blood plasma are as 4·44 to 0·259, and Fahr (1909) has made it practically certain that the sodium of frog's muscle is contained only in the intercellular lymph, etc., the muscle cells themselves containing no sodium at all. Such facts necessitate in this case also the existence of a membrane impermeable to salts.

According to Meigs and Ryan (1912, p. 411), however, the salts of smooth muscle are present in a non-diffusible form, and these authors do not admit the presence of a semi-permeable membrane. The evidence given is, I think, not very convincing. Smooth muscles are stated, when immersed in hypotonic saline solution, to gain in weight according to a different time law from that of striated muscle in the same conditions. This fact is readily to be accounted for by a different amount of imbibition in the two cases. Imbibition may play a relatively important part in smooth muscle, although as we have seen above (page 116), it plays only an insignificant part in the case of striated muscle. Water taken in by imbibition is not, of course, active osmotically, so that in order to balance a given external osmotic pressure, more water must be taken in per unit time if part of it is inactive. Again, it is said that, if smooth muscle is immersed in an isotonic solution of cane-sugar, it gains weight much more rapidly than striated muscle does; but we shall see presently that cane-sugar is by no means an innocuous substance for many cells, and the more rapid gain of weight is what would be expected if a certain amount of imbibition were taking place. It appears also that, when smooth muscle is cut across, its potassium content diffuses out very slowly; the possibility of adsorption, or the formation of a new membrane on the cut surface, is not taken into due consideration. These observers also regard the loss of potassium phosphate by ordinary muscle in activity and its replacement as inconsistent with a semi-permeable membrane. But, admitting the loss of phosphate, we shall see later that there is an increase of permeability in the excited state and it may well be that the passage of salts takes place at this time.

There are many other facts, of interest also on their own account, which prove an impermeability to crystalloids.

Bethe (1909) found that medusæ, floating in sea water stained with neutral red, stored the dye in their cells with the orange-red colour which it has in a solution of neutral reaction. If hydrochloric acid were added to the water, so as to give the dye in it a cherry-red colour, it was found that no change was produced in the tint of the cells for several hours; in fact, acid paralysis might be caused, but no change in the colour of the cells could be seen, until they were dead. The same thing was noticed with sodium hydroxide; the cells did not become yellow, the colour of neutral red in alkaline solution.

From the experiments of O. Warburg (1910) on the eggs of a sea urchin, the same fact, amongst others, was clearly made out. In this case, it was shown that the absence of change of colour was really due to non-entrance of alkali, and not to some fixed state of the dye making it inert to alkali, by taking an alkali to which the cell membrane is known to be permeable, such as ammonium hydroxide, in which case the colour became yellow almost instantly.

The objection may be made that the chemical or adsorption compound of the dye with cell structures may be less sensitive to sodium hydroxide than to ammonium hydroxide. This has been dealt with by Newton Harvey (1913), who has shown that the adsorption compounds of neutral red with various proteins, with lecithin, etc., are affected by these two alkalies in exactly the same concentration. Moreover, when the sea urchin eggs are made actually permeable to sodium hydroxide, by the action of sea water saturated with chloroform, this alkali changes the neutral red in the cells just as readily as ammonium hydroxide does.

An important fact emerges from the above experiments of Bethe and Warburg. That is, that acid and alkali can produce their characteristic effects without entrance into the substance of the cell. This question will be referred to again later.

Jacques Loeb (1909), in investigating the effect of acids on the formation of the fertilisation membrane in the eggs of the sea urchin, found that this effect was not in proportion to the strength of the acids, but to their permeability or

lipoid solubility. In fact, the mineral acids were far less active than the fatty acids.

Hustin (1912, p. 334), in perfusion of the pancreas with saline solutions, found that, if these were hypotonic with respect to the normal blood, the concentration was *increased* by passing through the blood vessels of the gland. If hypertonic, the concentration was diminished. The explanation on the basis of semi-permeability of the gland cells is simple; these cells would take up water from a hypotonic solution in order to equalise their osmotic pressure to it and give up water to a hypertonic solution. No satisfactory explanation is apparent on any other view. No change takes place in the composition of the perfused fluid if the cells have been killed by sodium fluoride, so that their semi-permeability is abolished.

The ratio of the *sugar content* of blood corpuscles to that of the plasma is very variable, although as a rule higher in the plasma than in the corpuscles. The addition of glucose to the blood sometimes raises the content of the corpuscles, sometimes not (Höber, 1912, 1). It is difficult to give an explanation of these facts. It seems that the conclusion must be drawn that the corpuscles are capable of being made permeable or impermeable to glucose, but that their usual condition is that of impermeability.

At this point it is well to call attention to the remarks justly made by Höber (1911, p. 244) to the effect that it is impossible to account for the constant difference in the ratio of potassium to sodium in the blood corpuscle and other cells compared with that in the plasma, which bathes them, except on the hypothesis of *complete* semi-permeability. If these salts were able to diffuse out, however slowly, equilibrium must result sooner or later, unless the extremely improbable assumption be made that the corpuscles and other cells obtain a continuous supply of salts from some source other than the blood and that the latter is able to get rid of them as fast as they pass in.

We now come to the *third* set of facts proving the semi-permeability of cells towards salts, namely, those connected with the electrical conductivity of cells. A few preliminary words of explanation are desirable.

When an electrical current is passed through a solution of a salt by means of wires dipped into it, the transport of electricity from one wire to the other is effected by means of atoms or molecules, each carrying a definite amount. These along with their charges, which differ according to the valence of the carrier, are called *ions*. The unit charge, carried by a univalent ion, is known as an *electron*. A bivalent ion carries *two* electrons and so on. Imagine a flock of sheep at one side of a field and that they start to run to the other side; the amount of wool (=electricity) which arrives at the other side in unit of time depends on the number of sheep and on the freedom of the course. Suppose that there are a number of square pens in the middle of the field, each fenced round and separated from the neighbouring pen by a narrow interval, the number of sheep now getting across in unit time will be much less than before, because they have to wait for each other to get through the openings, or rather, they obstruct one another in their efforts to get through. We may say that less wool passes across per unit time, or in electrical terms, the conductivity is less. Further, matters would not be improved if the closed pens were full of sheep, since these sheep would not be able to help in the transport. On the other hand, suppose the cross-fences were removed, the enclosed sheep could get out and cross the field, while the originally free sheep would have as clear a course as if no pens were there.

Living cells, as regards the transport of electricity, are like the enclosed pens with sheep in them and are in the same way obstructive to the passage of ions by filling up part of the channel. Whereas, if we make their membranes permeable to salts, the resistance is removed. This fact, in the case of the blood corpuscles, was described in detail by G. N. Stewart (1897) and made the basis of a method of determining the relative proportion of corpuscles and plasma in blood (1899).

Osterhout (1912) also finds that living cells of *Laminaria* are impermeable to the salts of sea water, as shown by their taking no part in the conduction of an electrical current. They are made conductors by any agent which kills the protoplasm, such as heat, chloroform, and so on. Their permeability also can be changed reversibly, as will be seen later. M'Clendon (1910, p. 255) finds that the eggs of sea urchins massed together have a conductivity greatly inferior to that

of sea water, and regards the fact as being due to impermeability of the cell membrane to ions.

The fact that a membrane being impermeable to salts makes it a non-conductor is shown in an interesting way in the method used by Morse and Horn (1901) in preparing copper ferrocyanide cells. By passing an electrical current through the membrane from copper sulphate outside to potassium ferrocyanide inside, the imperfect places are filled up and the resistance of the membrane gradually rises; for example, in one case reported by Berkeley and Hartley (1906, p. 487) the resistance of a membrane rose from 2,700 ohms to 300,000 ohms.

Although the resistance offered by living cells to the passage of a current of electricity is explained simply and satisfactorily by the existence of a membrane which is impermeable to salts, it must not be overlooked that other explanations have been advocated. It is very difficult or impossible to prove experimentally that cells are complete non-conductors, owing to the practical impossibility of removing all external electrolytes from the solution bathing them, except by means which affect the normal state of the membrane. We cannot, therefore, make the definite statement that cells are actual non-conductors, so that there is a possibility that their high resistance may be due to the presence of electrolytically dissociated colloids, enclosed in a membrane impermeable only to colloids. This circumstance would, as we shall see more in detail later, oppose the passage of a current in one direction entering the cell, and in the opposite direction on leaving it, since the one ion is imprisoned. It may be objected to this view that the presence of such colloids in the blood corpuscles has not been proved.

If the electrolytes within the cell were combined with the cell-proteins, in the form of non-dissociated salts, they would be non-conductors, since ions only can convey a current. But there is no experimental evidence to warrant an explanation of the facts of the case on such an assumption. Reasons have also been given previously to show that adsorption is insufficient as an explanation, since an adsorption compound exists only in presence of free electrolytes in the liquid phase with which it is in contact. Free electrolytes must, therefore, be present in the interior of living cells. Their existence in that situation has been, in fact, demonstrated experimentally by Höber in two ways.

The first of these (1910, 2) depends upon the fact that the capacity of a condenser is increased when a conducting stratum is introduced into the dielectric between the plates, and the amount of the increase is proportional to the conductivity of the stratum. It will be clear that there is no question of ions being able to leave the cells in such a case. By this method, the internal conductivity of blood corpuscles, after repeated washing with cane-sugar solution, was found to be about the same as that of a decinormal potassium chloride solution.

The second method (1912, 2) is founded on an experiment by J. J. Thomson (1895). A conducting body, placed in the axis of a coil of wire through which a rapidly-alternating current is passed, diminishes the strength of this current by damping the vibrations, and it does this in proportion to its own conductivity. By this more sensitive method, the content of blood corpuscles in free electrolytes showed itself to be equal to that of a 0·1 to 0·4 per cent. solution of potassium chloride. The method was afterwards improved (1913) so as to require less material, and at the same time to be increased in sensibility. Frog muscles were also investigated by its means, and found to have an internal conductivity equal to 0·1 to 0·2 per cent. sodium chloride.

Comparing this number with the analyses of Fahr (1909), we note that a part of the salts must be adsorbed on the colloid surfaces, or in chemical combination in some form other than a dissociated salt, so that this part does not contribute to the conductivity, which is less than what would be given by the total salts of Fahr's results. It is also of interest to note that the above value of the internal conductivity of muscle cells was obtained after six hours' soaking in isotonic cane-sugar, so that the membrane had not allowed the electrolytes to escape from the cell.

It has been suggested by Roaf (1912, i. p. 146), as indicated above, that the properties of a colloidal salt, in allowing a current to pass through a membrane in one direction only, might account for the high resistance of cells, without the necessity of a membrane impermeable to crystalloids. I showed indeed (1911, ii. p. 242) that if a salt, of which one ion only is in the colloidal state, be separated

from water by means of a parchment paper membrane, and an electrical potential difference established by placing electrodes, one inside, the other outside the membrane, then it depends on the sign of the electrode compared with that of the colloidal ion whether a current passes or not. Suppose we have a sodium salt of a colloidal acid, such as caseinogen or Congo-red, and that the electrode in this solution is the positive one or anode. The current must pass through the membrane from inside to outside; that is, positively charged ions must pass through to the negative electrode and negative ions from outside to inside and be discharged there; unless this can happen, no current will pass. Now, sodium ions can freely pass through the membrane and the opposite negative ions are already inside, so that current will flow when the internal electrode is the anode. On the contrary, if the outer electrode is the anode, in order that a current shall pass, the negative ions must reach it. This cannot happen, since there is an impassable barrier between them and the electrode.

Such conditions would clearly account for the resistance of cells to the passage of currents. The boundary surface on the one side of the cell would oppose currents in one direction, and that on the other side, those in the opposite direction. They would appear to be non-conductors. But it is to be remembered that this state of affairs holds only as long as the colloidal ion is the only one available of the right sign. If any diffusible ion is present, the current will pass by means of it, and we know that there are in the cells inorganic ions of both signs. A high resistance might be accounted for by the existence of most of the inorganic constituents of the cell in the form of salts with colloids, while the non-colloidal salts of the cells and the plasma of the blood were freely diffusible. But, as we have shown (page 120), if this were the case, the ratio of the different cations, say of potassium and sodium, must be the same inside and outside the blood corpuscles, and this is not what is actually found.

FUNCTIONAL CHANGES IN PERMEABILITY

It appears from the preceding section that we must regard the surface membrane of cells, at all events in the condition in which they are usually investigated, as being impermeable both to colloids and to the majority of crystalloids.

There are, however, certain substances—ammonium salts, urea, glycerol, alcohol, etc.—to which the membrane is more or less permeable at all times. When placed in hypertonic solutions of these, there is a preliminary plasmolysis or shrinking of the cell, greater or less according to the diffusibility of the solute, but this disappears as the concentration becomes equal on the two sides of the membrane.

On the other hand, we know that it is necessary for cell processes that such things as glucose and amino-acids, which are usually unable to pass the membrane, should get into the cell. For this reason certain recent work, showing that it is possible to produce reversible changes of permeability without killing the cell, are of great importance.

Osterhout (1912) showed, as already stated, that the cells of *Laminaria* are impermeable to the ions of sea water, when immersed therein. But, if immersed in pure sodium chloride of the same conductivity (and temperature) as sea water, their conductivity rapidly rises, until they oppose very little more resistance to the passage of the current than the salt solution itself does. If the exposure to the sodium chloride has not been too prolonged, the normal state of the cells is recovered on return to sea water.

It may be remarked, in passing, that this fact seems impossible to account for on the view of the membrane being only semi-permeable as regards colloids; for it would be necessary to assume that it becomes permeable to colloids under the action of sodium chloride; in which case the protoplasmic substance of the cells would diffuse away and no recovery be possible on replacing in pure sea water.

Lillie (1909) found that the larva of *Arenicola*, if placed in pure sodium chloride, isotonic with sea water, constricts up and the pigment contained in its cells diffuses out freely. This pigment is soluble in water, and does not appear to be in colloidal solution. The addition of one volume of 0·5 molar calcium chloride

to 24 volumes of the 0·5 molar sodium chloride prevents the contraction, and also the loss of pigment.

Fluri (1909), again, found that three days' immersion in 0·01 per cent. solution of aluminium sulphate makes *Spirogyra* permeable for most salts as well as glucose, and that the effect can be removed, so that the cells become normal again, by return to pure water.

Newton Harvey (1911, p. 546) states that a sodium salt makes the membrane of *Spirogyra* and of *Elodea* permeable to sodium hydroxide, to which, as we have seen in Warburg's experiments, it is normally impermeable.

Another fact which may be mentioned is that M'Clendon (1912, i. p. 296) found that the eggs of *Fundulus* lose magnesium in pure sodium chloride solutions.

Siebeck (1913) showed that frog's muscle, if immersed in isotonic potassium chloride, swells, showing that the action of the potassium salt is to diminish or abolish the impermeability to potassium, which the muscle normally possesses in the presence of sodium and calcium.

Wächter (1905) showed that the passage of sugars from the cells of the onion was inhibited by the presence of potassium nitrate.

Osterhout (1910) shows that the root hairs of *Dianthus barbatus*, grown in distilled water, contain no crystals of calcium oxalate. If the water be changed for a solution containing calcium salts, the crystals soon make their appearance. They may easily be detected by observation between crossed Nicols in the polarising microscope.

Gérard (1912) found that, on feeding animals with excess of potassium salts, the blood maintains its constant composition, while the cells of the tissues lose sodium.

These various facts are given in order that the reader may grasp the fact that the cell membrane is capable of changes in its permeability.

Instructive experiments may easily be made with slices of the root of the red beet. It will be found that the pigment does not leave the cells when immersed in tap water. (It is well to rinse the slices previously for a minute or two in tap water in order to remove the contents of the cells which have been injured in the process of cutting the slices.) If, on the contrary, they be placed in pure sodium chloride of 0·31 molar (= 1·82 per cent.) strength, which is about isotonic with the cell contents, the pigment will gradually come out. Addition of 0·17 per cent. of calcium chloride to the pure sodium salt prevents this effect. It is convenient to take 3·64 per cent. solution of sodium chloride and to dilute it with an equal volume of water or of 0·34 per cent. calcium chloride as the case may be. Many other experiments on permeability may be made with the red beet; chloroform, bile salts, soap, warming to 50°, all cause loss of pigment, but in most cases the cells are killed. If it be desired to make quantitative experiments, the cane-sugar, which escapes along with the pigment, may be estimated by an appropriate method. In this case, the slices to be compared must, of course, be of equal dimensions.

It seems evident from the various instances quoted that calcium must produce some change in the properties of the cell membrane and of such a kind as to make it less permeable, and that sodium has the opposite effect. Osterhout (1912, ii. p. 114), in fact, states that visible effects are to be detected under the action of calcium. This antagonistic nature of calcium and other ions is of much importance and will require treatment in Chapter VII.

A matter of some practical importance is the *action of cane-sugar* on the cell membrane. For the investigation of the effect of various salts, it is necessary to have cells suspended in an isotonic solution of a non-electrolyte. Now, while cane-sugar appears to be the least injurious, and at the same time convenient, especially if not in contact with the cells for too long a time, there are several facts which show that it increases the permeability of the membrane if the contact is prolonged. Bethe (1908, p. 560) found that the contractions of medusæ were slowed if one part of isotonic cane-sugar was added to nineteen of sea water. Magnus (1904, p. 131) found that the movements of the excised intestine in Ringer's solution were weakened by the addition of cane-sugar above 0·02 per cent. Küster (1909) noticed that, on plasmolysis of the cells of the onion in hypertonic cane-sugar, the protoplasm broke up into separate clumps and that, on placing in water, these clumps did not fuse together again, while the surface membrane seemed to be fixed or coagulated. According to Bang (1909, p. 263) blood corpuscles give up salts to isotonic cane-sugar, after prolonged contact with it. Muscle, on the contrary, is relatively resistant to the action of cane-sugar, giving up in twenty-two hours to repeated changes scarcely more salts than those contained in the spaces between the cells (Fahr, 1909). Overton (1902, ii. p. 349) showed that a muscle, which had lost its excitability by lying in cane-sugar solution, owing to removal of sodium salts from between the cells, quickly regains its excitability when placed in sodium chloride, so that no permanent injury is inflicted.

A further practical point of some importance is that, when a substance is found to penetrate into a cell, the conclusion must not hastily be drawn that the cell is normally permeable to this substance. The experiments of Osterhout (1912), in which the cells of *Laminaria* were found freely permeable to sodium chloride when this salt was present alone, but impermeable to it when calcium was also present, are sufficient to prove the contrary. In fact, statements regarding permeability to any particular substance can only be held to be valid when the proof is given that the membrane is in its normal state, a proof that is not always given, and one which, as must be confessed, it is not always easy to give.

There are certain other substances, in addition to electrolytes, which produce changes in permeability. The most important of these are those known as anaesthetics or narcotics and will be discussed in a succeeding section of this chapter.

Certain functional states of the cell are known to be accompanied by changes of permeability; the state of excitation produced by stimuli in contractile tissues appears to be accompanied by increased permeability to electrolytes; this will be discussed later.

Lepeschkin (1908) finds that the permeability of plant cells is increased by exposure to light. The question was worked out further by Tröndle (1910), especially with respect to the relation between the amount of change and the intensity of the illumination. The bearing of this fact on the explanation of the movements which take place under the action of light is obvious. Diminution in permeability produces a fall in the concentration of osmotically-active substances in the cell, the osmotic pressure and turgor consequently fall in value, so that opposing forces are able to bend the side of a stem exposed to light. Hence the heliotropic curvature. V. H. Blackman (1918) also finds that light causes increase of permeability in the pulvinus of the sensitive plant, described on page 431 below.

Again, the great variation in the relative concentration of sugar in the blood corpuscles and the plasma, and the manner in which changes in the concentration in the plasma affect that in the corpuscles, serve to show that the permeability of blood corpuscles is not a fixed and unalterable thing. The following data from a paper by Höber (1912, i.) will illustrate the point:—

An increase of glucose concentration in the blood was produced in various ways, adding glucose to shed blood, and determining the distribution between plasma and corpuscles after standing, giving adrenaline to the living animal, extirpation of the pancreas, or a large amount of glucose introduced into the stomach.

	Glucose, per Cent.		Ratio.
	Plasma.	Corpuscles.	
Blood of dog + glucose	0·572	0·106	5·4
,, after 30 minutes	0·576	0·099	5·8
Blood of dog + glucose	0·793	0·074	10·7
,, after 39 minutes	0·793	0·112	7·1
Dog, normal	0·125	0·049	2·5
,, after adrenaline	0·339	0·078	4·4
,, ,, 40 minutes later	0·413	0·059	7·1
Dog, after pancreas extirpation	0·621	0·192	3·23
Rabbit, after 20 g. glucose in stomach	0·214	0·249	0·86
Rabbit, after 20 g. glucose in stomach	1·017	0·293	3·5

There is clearly no question of parallelism, as would be the case if the corpuscles were always permeable to glucose; neither does the content of the corpuscles remain constant, as would be the case if they were always impermeable. As a rule, rise in the content of the plasma is associated with a rise in that of the corpuscles, but not in invariable proportion. The facts suggest the possibility that the normal semi-permeability of the membrane to glucose is connected with a particular difference of concentration on the two sides, but that the actual value of this difference may be changed by other influences. The membranes may be, as it were, tuned to different concentrations of glucose by the action of other substances. Similar conditions may perhaps apply to cells in general, but the data as yet available are not sufficiently decisive.

The action of electrolytes on the permeability of the membrane suggests that *electrical forces* play a part in the phenomena. The relation to precipitation of colloids will occur to the reader. It seems also possible that the presence or absence of an electrical charge on the membrane itself may be of importance in determining the permeability to ions. Suppose that a membrane has a negative charge, it would, to a certain extent, oppose the passage of electro-negative ions. Certain experiments by Girard (1910, p. 479) seem to support this view. A membrane of gelatine allowed magnesium chloride to pass more freely when given a positive charge by the presence of a trace of acid. The change produced in the structure of the membrane, however, must be taken into consideration. In any case, it is difficult to see how the presence of an electrical charge could exercise a permanent influence on the distribution of an electrolyte between the two sides of a membrane, although the time taken to attain equilibrium might be affected, a factor of importance in rapid changes of state. The experiments of Mines (1912) on the production of potential difference will be referred to in Chapter XXII.

The *work of Overton* (1899, i. and ii.) has shown a striking correspondence between the nature of a dye, as the salt of a colour-acid or a colour-base, and its passage into cells. While the cell membrane is impermeable to the former, it is readily permeable to the latter. The fact is brought by this investigator into relation with lipoid solubility and the lipoid nature of the membrane, a question to be discussed presently. Here, we may direct attention to the fact that these two classes of dyes, or the coloured ions into which they dissociate, have opposite electrical charges. The so-called "acid" dyes, that is, those in which the coloured part of the salt is the acid radical, are electro-negative, while the "basic" dyes are electro-positive, a fact which would undoubtedly have much influence on their adsorption by constituents of the membrane and of the cell itself. In fact, Endler (1912) has shown that the rate at which the diffusible dyes enter the cell is greatly affected by the presence of various electrolytes and brings the fact into relation with changes in electric charge, although it does not seem quite clear whether the effects described by him are not rather of a "lyotropic" origin.

Hardy and Harvey (1911, p. 220) find that unicellular plants and animals possess, as a rule, a surface charge, which varies with functional activity. This latter fact is shown by the circumstance that different individuals of the same species in a mixed culture were found to migrate in an electric field at different rates. Red blood corpuscles, on the other hand, have a markedly uniform rate of migration and may be regarded as having very slight chemical activity, although living. The activity they possess is also very uniformly distributed between individuals.

From the work of Lillie (1917, p. 49) it appears that the possibility of the cell membrane becoming completely impermeable, or "waterproof," under certain conditions, must be taken into account.

THE CONSTITUTION OF THE CELL-MEMBRANE

To begin with, we must remember that the film covering the outer surface of protoplasm, or, in fact, any surface where it is in contact with another phase, is not of such a nature that it can be separated off, even optically, from the rest of the cell. After death, under the action of toxic substances, it seems that a distinct membrane may be visible. There are, of course, membranes covering whole organs which can be separated from the cells beneath them, such as the interesting one on the barley corn, whose properties have been investigated by Adrian Brown (1909). Such membranes play an important part in physiology, but are to be distinguished from those with which we are immediately concerned.

Suppose that a mass of protoplasm, such as an *Amœba*, is immersed in water. By the principle of Willard Gibbs, any constituent of the protoplasm which lowers surface energy will be concentrated at the interface between the two phases, forming already a kind of membrane. Further, as shown by Ramsden (1904) and described on page 55 above, many of the substances present in cells, especially the proteins, suffer a kind of coagulation when subjected to such concentration. Now, substances of a fatty nature, the so-called lipoids, such as lecithin, and the fats themselves, are normal constituents of cells and, as we saw in Chapter III., have a particularly powerful action in decreasing surface energy and will naturally take a large share in the formation of a membrane of the kind in question.

An interesting experiment by Nägeli (1855, i. pp. 9 and 10), discussed also by Pfeffer (1897, i. p. 92, and 1877, p. 127, etc.), shows that such membranes are formed on any free protoplasmic surface. A root hair of *Hydrocharis* (a water plant with relatively long root hairs, which are processes of the root cells themselves) is placed under a cover-glass in a solution of a dye, such as aniline-blue, to which the normal cells are impermeable. The root hair is then crushed by pressure and, from the places where the cell wall is torn, masses of protoplasm exude and form into little balls. These balls show similar osmotic phenomena to those of the entire cell. The protoplasm remains unstained by the dye. Kühne, also (1864, p. 39), describes the formation of a similar membrane on protoplasm pressed out from *Stentor*, a ciliate protozoon. Further observations will be found in Pfeffer's paper (1890, p. 193, etc.) and in that of Chambers (1917).

It seems probable that the observations of Kite (1913), in which solutions injected into the substance of certain cells, so as to form vacuoles, which behaved as if surrounded by a similar membrane to that on the outside of the cell, are to be explained by this formation of a surface condensation at the interface between the solution in the vacuole and the surrounding protoplasm.

It may be noted here that the clear surface layer of protoplasm, noticed in *Amœba*, leucocytes, *Mycetozoa* and similar organisms, and known as "hyaloplasm," also owes its origin to surface forces. When the cell changes in dimensions, as by taking up or losing water, it is found that the thickness of the layer of hyaloplasm does not change, so that its total volume must have altered. It is constantly maintained so as to extend to a particular depth below the surface. It is not to be thought that this clear layer is the cell membrane itself, to which the semi-permeability is due. This is to be seen from the fact that a dye, which is unable to enter a cell, is stopped before it reaches the hyaloplasm, which remains unstained, like the rest of the cell (Höber, 1911, p. 59).

The new formation of a cell membrane on fresh surfaces of protoplasm, referred to in the preceding paragraphs, occurs only in the "living" state, although, under certain conditions, it remains intact after the death of the cell, as shown by the following experiment of Pfeffer (1877, p. 136). A root hair of *Hydrocharis* is mounted in an isotonic solution of cane-sugar, placed under the microscope, and a trace of hydrochloric acid added. The protoplasm becomes granular and opaque, and its movement ceases, that is, the cell is killed. But if cherry juice or other dye, to which the normal cell is impermeable, be added, it will be seen that, although the cell is dead, the membrane remains impermeable, since the dye does not enter. But suppose that we now replace the coloured isotonic solution by a hypotonic one. The cell expands by taking up water, but, contrary to what happens in the living cell, the membrane does not expand also, so that it gives way at one place or another; the defect is not made good, the dye enters and slowly stains the whole of the protoplasm. One must not, however, hastily draw the conclusion that this semi-permeable membrane, after the action of hydrochloric acid, is the same thing as the natural one. The experiment merely shows the possibility of producing a membrane similar to the natural one in its properties and situation.

Under certain circumstances the existence of an actual membrane can be made visible, although there is no proof that the membrane was in existence in the living cell in the same state as that seen. As already said, a membrane similar to that

on the outer surface of the cell protoplasm exists also on the surfaces of the vacuoles enclosed within it.

De Vries (1885) takes *Spirogyra* and plasmolyses by immersion in 10 per cent. potassium nitrate coloured with eosin. After about an hour, the cells die, become stained and the red shrunken protoplast lies in a rose-coloured liquid situated between it and the cell wall. The vacuoles alone remain unstained and sometimes shell out of the cell as colourless balls, which slowly take up the dye. In this process it is seen that the surface layer becomes very deeply stained before the dye penetrates to the interior liquid.

At the beginning of the present section, it was pointed out that contents of the protoplasm, capable of lowering surface energy, are concentrated on the surface and are, in all probability, the origin of the cell membrane. The experiment just described suggests a further important point. The interface between two phases may be regarded as belonging to both phases, so that constituents of *both* phases will be concentrated there if they lower surface energy. This circumstance does not much concern the protoplasm of organisms like *Amœba* or the cells of plants, for the most part, where the external phase is nearly pure water, but is of considerable importance in the higher animals, where the fluids in contact with the cells are of a highly complex composition. The difference seen in the experiment of De Vries, quoted above, between the outer cell membrane, which has been killed, and allows potassium nitrate and dye to pass freely, and the membrane of the vacuole, which is not for some time made permeable to them, suggests that the composition or structure of the membrane in contact with the contents of the vacuole is not the same as that of the outer cell membrane. This difference is probably due to the presence of substances in the vacuole, which contribute to the formation of the membrane.

It will be noticed that the view here taken as to the nature of the cell membrane implies that it is a variable thing as regards its composition, since this depends on the substances present in the protoplasm of the cell, and in the surrounding medium, at any given time. In a certain sense, it is, indeed, a part of the protoplasm, so that it is not to be wondered at that its permeability is capable of change with varying functional states of the cell. The fact that it is readily formed is shown by the experiment of Nägeli, described above, where a new surface of protoplasm becomes rapidly covered with a membrane, having apparently the same properties as concerns permeability as the original one. That it can be reabsorbed is shown by the facts that pseudopodia of protozoa will fuse together, and that a number of amœboid organisms, as in *Mycetozoa*, will unite to form a plasmodium. In the above sense, we may accept the view taken by Höber (1911, p. 264), that the cell membrane is a living structure. In the way in which I regard it, it may be said to be a local concentration of integral parts of the cell protoplasm.

There is a certain amount of optical evidence of the existence of something on the surface of protoplasm distinct from the inner mass. Gaidukov (1910, p. 51, and Fig. 3B on plate v.) describes, in a germinating spore of a mycetozoon, the appearance under dark ground illumination of a reticulated appearance on the surface of the protoplasm; this network had a violet colour, while particles in the endoplasm had a yellow colour. Osterhout (1912, ii. p. 114), also, saw an obvious change on the surface of protoplasm under the action of calcium. It is well to be cautious in the interpretation of these phenomena, owing to the possibility of diffraction effects.

The question of the *chemical composition* of the cell membrane has excited much discussion. Since lipoid substances, with cholesterol, are universal constituents of protoplasm, while they possess in a marked degree the power of lowering surface tension, it is practically certain that they must form an important part of the membrane. Now, Overton (1899) has advocated the view that the limiting membrane of the cell is essentially of a lipoid nature, and has supported this hypothesis by a large amount of powerful evidence, which it is important, as well as instructive, to examine somewhat closely. It is, in the first place, a very remarkable fact that, in the case of cells of the most various kinds, in the state in which they are usually investigated, the substances which easily obtain entrance into the cell are just those which are soluble in lipoids. In view of certain facts, to be spoken of later, it is, perhaps, more correct to say, that those substances in which lipoids are soluble, such as alcohol, chloroform, benzene, etc., and those which are themselves soluble

in liquids which dissolve lipoids, such as urea, fatty acids, some ammonium salts, etc., are found to penetrate the cell membrane. Those to which the membrane is impermeable are not dissolved by lipoid solvents; such are sugar, amino-acids, inorganic salts, mineral acids, etc. We note, for example, that sodium hydroxide, insoluble in benzene, does not penetrate, while ammonium hydroxide, which is soluble in benzene, readily does so. But it will doubtless occur to the reader that these two bases differ in many other ways besides that of solubility in benzene.

Again, Loeb (1909) showed that the lower fatty acids are more effective in modifying certain cell processes, such as those involved in the fertilisation of ova, than the mineral acids are. The fact can be explained on the ground of the "lipoid-solubility" of the former.

The aniline dyes were made extensive use of by Overton (1899, ii.) to test the hypothesis, and it was found that those soluble in lipoids, that is, the salts of colour bases, passed into the cell, while those not soluble, salts of colour acids, did not. The meaning to be attached to the phrase "lipoid-solubility" in this connection will appear hereafter. We may note also that the "basic" dyes which enter, are uniformly electro-positive as regards the coloured substance to which we direct our attention, while the "acid" dyes are electro-negative, so that lipoid solubility cannot be adduced as the only difference between the two classes. Further, just as remarked above with reference to the hydroxides of sodium and ammonium, it cannot be held that "lipoid-solubility" is the only difference between acetic and hydrochloric acids.

In fact, a layer of benzene shows the same selective permeability in respect of organic or weak bases and acids, on the one hand, and strong inorganic bases and acids on the other hand, as the cell membrane does, but no one supposes that this membrane is composed of benzene. Benzene, however, does not dissolve even the "basic" dyes, although solutions of certain lipoids in chloroform, etc., appear to do so. It will be seen presently, however, that there is strong evidence that this is really an adsorption on the surface of the lipoid, which is only in colloidal solution.

Notwithstanding what has just been said, it seems from the work of Overton that we must admit that "lipoids" play an important part in the properties of the cell membrane, although we shall see later that it is impossible to assign the total composition of the cell membrane to them. Moreover, we shall find that there are difficulties in looking upon them as solvents in the ordinary sense.

At this point, then, we may profitably consider some of the chemical and physical properties of the cell constituents to which the name "*lipoids*" has been somewhat loosely applied.

We find sometimes that all those substances extracted by alcohol are called lipoids. This is clearly calculated to cause confusion. Glucose, urea, free bases, such as choline, may be mentioned as being soluble in alcohol, but not of a lipoid nature. Overton himself includes cholesterol, although, strictly speaking, the name should be restricted to substances chemically related to the fats proper. For the present purpose, perhaps, it may be allowed to remain in the class of lipoids, owing to the similarity of its physical properties.

The simple ordinary fats, glycerol esters of both saturated and unsaturated higher fatty acids, are common constituents of the cell, but the most interesting are those complex fats, to which the name "lipines," with its derivatives, has been given by Leathes (1910). Lipines themselves are compounds of fatty acids with a nitrogen-containing group, but contain no phosphorus nor carbohydrate. Phospholipines contain phosphorus in addition, and are sometimes called "phosphatides," while "galactolipines" contain no phosphorus, but a carbohydrate group, galactose, and correspond to the cerebrins or cerebrosides of some authors. The most familiar of these lipoids is the phospholipine, lecithin, of which the formula is usually given thus:—

$$\begin{array}{l} CH_2.O.OC.C_{17}H_{33} \\ CH.O.OC.C_{15}H_{31} \\ CH_2.O-P=O \\ \qquad \diagup \quad \diagdown \\ \quad OH \quad O.C_2H_4 \quad\ \ .CH_3 \\ \qquad\qquad\qquad\qquad \rangle N.CH_3 \\ \qquad\qquad\ \ HO \quad\ \ .CH_3 \end{array}$$

It may be looked upon as glycero-phosphoric acid combined up with one molecule each of oleic and palmitic acids, on the one hand, and with choline, a base, with the constitution of a tertiary amine, on the other hand. It is to be remembered, however, that other fatty acid radicals may take the place of oleyl or palmityl, and other bases the place of choline.

The physical properties of this substance are the most important in the present connection, and they are somewhat remarkable. It is a soft, waxy, substance, soluble (probably in colloidal form) in chloroform, benzene, oil, and alcohol, rather less so in ether; insoluble in cold acetone or ethyl acetate. Placed in contact with water, it tends to disperse, assuming the so-called "myelin" forms, like the pseudopodia of amœboid organisms. If shaken up with water, it forms a colloidal solution of the emulsoid type, in which the internal phase consists of lecithin containing "imbibed" water.

Although the physical properties are the most striking, the chemical composition suggests important functions of a chemical nature, but what these are is at present very uncertain.

When alcoholic solutions containing lecithin and glucose or certain proteins are evaporated to dryness, it is found that ether takes up from the residue adsorption compounds of lecithin with glucose or protein, substances normally insoluble in ether. It was at one time supposed that these were definite chemical compounds, but it has been shown that the proportion of the constituents varies with that in the original mixture and is never definite. The cases of "jecorin," which contains glucose, and of "vitellin," have been already discussed (page 66 above).

The relationship of lecithin to water is of much importance as regards the cell membrane. This membrane, with very rare exceptions, is freely permeable to water. Now, the true fats are not so, while lecithin, as stated above, easily swells up in water, and is therefore permeable to it. But, as Nathansohn (1904, p. 640) points out, in this state it has lost its power of being a solvent for lipoid-soluble substances only; dry lecithin in solution in benzene dissolves the "basic" dyes only, but moist lecithin in benzene is also a "solvent" for the sulphonic acid dyes, to which the cell membrane is normally impermeable. Ruhland (1909, p. 34) prepared membranes of lecithin and cholesterol in the manner of Pascucci (1905), and found that no dye, "basic" or "acid," diffused through cholesterol at all. Neither did this happen through lecithin membranes, which were completely impermeable until saturated with water; when this occurred, as could be seen from the fall of the water column in the cell, both kinds of dyes began to come through.

Loewe (1912) has recently published important work on the physical chemistry of these "lipoids." Kephalin is a substance closely related in its composition to lecithin and present in considerable amount in brain. As already mentioned, lecithin forms an obviously colloidal solution in water, and Loewe shows that kephalin, even in chloroform or benzene, is also in the colloidal state. The solutions show the Faraday-Tyndall phenomenon and an illuminated cone under the ultra-microscope. In solution in chloroform, the raising of boiling point is too small to be detected, showing that the solute is in large aggregates, a fact also evident from vapour pressure measurements. Further, when swollen by the action of water, it becomes insoluble in ether. The reader will probably remember that, in the old Hoppe-Seyler method of extracting the lipoids from brain, it was necessary to dehydrate first with alcohol in order to make them soluble in ether. The meaning of this insolubility in ether will be apparent when it is remembered that presence of water does not affect true solubility in ether, such as that of picric acid, which is extracted by ether from its solution in water. Kephalin, then, is not in true solution in these various so-called "solvents" for lipoids.

This fact raises considerable difficulty in the interpretation of Overton's experiments with "lipoid-soluble" dyes and other substances. According to his view, a substance obtains admission to the cell because it is soluble in lecithin and similar substances. Take the case of methylene blue. This is insoluble, except to a minute degree, in chloroform, but, if kephalin be present in the chloroform, the

chloroform-lipoid phase becomes deeply coloured when brought into contact with a watery solution of methylene blue. The explanation given by the adherents of the lipoid-membrane theory is that methylene blue is more soluble in kephalin than in water and that the staining of the lipoid is due to a true solution of the dye in it. Now Loewe brings strong evidence against this interpretation of the fact. Suppose that the dye is dissolved in true solution; there is a certain ratio between its concentration in the water phase and that in the chloroform-lipoid phase, known as the "partition coefficient," and, if the molecular weight is the same in both solvents, this ratio will not vary with the concentration. Loewe finds, on the contrary, that the ratio varies very considerably with the concentration, but that it follows the parabolic law of adsorption, viz., the ratio varies as some power of the concentration. The exponent $1/n$ has values between 0·35 and 0·16, according to the particular lipoid used, kephalin, cholesterol, residual brain lipoids, etc. On the other hand, for each individual lipoid, the value is fairly constant. The conclusion drawn is that we are dealing with a case of adsorption, but it must not be forgotten, as Loewe appears to have done, that the partition between solvents also has an exponential ratio if the molecular weight of the solute is not the same in the two. Take, for example, acetic acid dissolved in benzene and in water; in the former the molecular weight is double that in the latter, owing to the association of two molecules together. In such cases, the exponent expresses the ratio of the molecular weight in the two solvents, so that it must be a whole number. Now, in the case of methylene blue in lipoid and water, a ratio of whole numbers can only be obtained by assuming a very large association in *both* solvents; a quite impossible degrée in fact as regards water, where, judging by its electrical conductivity, there is no association. It appears, then, that Loewe's interpretation is correct. Moreover, as this investigator points out, if the phenomenon is a partition owing to different solubility, the dye would readily be removed when the lipoid phase is put into contact with pure water. But this is not so, and the case is precisely similar to that of paper stained with the dye. It will be remembered that paper, owing to its negative charge, has a strong adsorptive power for electro-positive dyes and is in equilibrium only when a very deeply-stained paper is in contact with a very dilute solution of dye. So that, as Freundlich points out, very little dye is removed by pure water. Another fact observed by Loewe, which shows the staining of lipoid by methylene blue to be a surface condensation only, is that, if a mass of kephalin be placed in contact with a watery solution of methylene blue, the dye does not diffuse into the lipoid. Further, if a solution of dye, to which gelatine has been added in order to prevent mixing of the various layers, be covered with a layer of lipoid and over this water be placed, no dye passes into the water. Similar facts were noticed with regard to other substances supposed to be soluble in lipoids, such as narcotics, nicotine and tetanus toxin. As concerns other lipoids, cerebroside (a galactolipine) and the lipoid residue from brain after removal of kephalin and cerebroside, all behaved like kephalin. Cholesterol was found to obey the partition law, but dissolved very little dye. Thymol in chloroform was found to be partly in a colloidal form, partly in true solution, but obeyed the adsorption law and not the partition law. In this last case, apparently, only the colloidal particles took up the dye. There is, finally, another difficulty involved in the acceptance of the solubility partition theory. If we take a particular case of Loewe's, say the first on Table II. (p. 161 of his paper), we see that the final concentration of the dye is greater in the lipoid-chloroform phase than in the water phase. Remembering that the dye is practically insoluble in chloroform itself, the result means that the solvent power of chloroform for the dye has been raised by the addition of 0·5 per cent. of kephalin to at least that of water. If we compare this effect with the increase of the solvent power of alcohol for cane-sugar, produced by the addition of as much as 3·28 per cent. of water, which was found by Scheibler (1872) to be raised only to 0·36 per cent., we are compelled to admit the inherent improbability of explanation on these lines.

So far as Loewe's experiments go, it appears that a lipoid membrane, so far from being an assistance to the passage of "lipoid-soluble" substances into

the cell, is rather of the nature of a hindrance, since it holds fast the substances instead of passing them on. At the same time, the fact has to be explained why it is just these particular things that enter the cell so easily, although some property other than partition according to solubility will have to be brought into the account.

Before proceeding further, a few words may be said as to *cholesterol*. The ubiquitous presence of this chemically-inert substance is a remarkable fact and suggests that it must have some important part to play in the regulation of the mechanisms of the cell. In strictness, it is not a lipoid, although for convenience usually reckoned with them. In chemical constitution, it is the monatomic alcohol of a substance related to the terpenes; according to Windaus and Stein (1904), the complex terpene in question is methyl-isopropyl-phenanthrene. The most familiar terpenes are the essential oils of plants, cymene, for example, from oil of caraway seed and from oil of eucalyptus is methyl-isopropyl-benzene. It is of some interest to find in the animal a representative of this class of substances so widely spread in the vegetable kingdom. Cholesterol is soluble in ether, benzene, chloroform and fats; insoluble in water and in cold alcohol. In the work of Loewe, above mentioned, it was found that it could take up lipoid-soluble dyes to a very small degree only and apparently in accordance with the partition law and not with that of adsorption.

Although it is necessary to hold that lipoids form a part of the constituents of the cell membrane, there is reason to doubt that they are the sole substances taking part in it. For one thing, it is very difficult to understand how the permeability is capable of regulation by processes occurring inside and outside of the cell unless the membrane has a very complex composition. There is, also, more direct evidence that a more complex structure than a mere lipoid one is concerned, as we shall see presently. But, whatever explanation may be given of the fact, it seems certain that cells are always permeable to substances soluble in lipoid solvents, while being only at times permeable to those not so soluble, such as sugars, amino-acids and most salts. There are, as it were, two kinds of permeability, of which the latter one alone is subject to functional change.

The presence of more than one constituent in the case of the membrane of the red blood corpuscles is shown by the experiments of Ryvosh (1907) on hæmolysis by saponin. This glucoside causes the corpuscles to break up by a kind of solvent or dispersive action on the cell membrane. It has remarkable powers of being adsorbed at interfaces between phases, driving out most other substances from this situation. At the same time, it is difficult to demonstrate that it lowers surface tension to any considerable degree. It is probable that this difficulty arises from the fact, discovered by Ramsden (1904), of the formation of a rigid film at the surface where its solution is in contact with another phase. We have seen that hæmolysis is also brought about by mixing the blood corpuscles with a hypotonic solution. The phenomenon in this case is due to swelling by osmosis. Now the corpuscles of different animals have a different relative power of resistance towards these two methods of hæmolysis, and in such a way that the corpuscles of some animals require the difference between their own osmotic pressure and that of the hypotonic solution, in order that hæmolysis may occur, to be greater than those of other animals. Also those of certain species require a higher concentration of saponin than in the case of other species. The important point is that the more resistant a particular kind of corpuscle is towards saponin, the more sensitive it is to a hypotonic solution and vice versa. The two series below illustrate this fact, the most resistant species in both series being at the top of the left-hand column:—

Hypotonicity.		*Saponin.*	
Guinea Pig	Grey Mouse	Sheep	Pig
White Rat	Cat	Goat	Grey Rat
Dog	Ox	Ox	Dog
Grey Rat	Goat	Cat	White Rat
Pig	Sheep	Grey Mouse	Guinea Pig

The rabbit alone of all the animals tested fails to come into the corresponding place in the two series.

It is evident that the constituent acted on by saponin is of a kind different from that which gives way when distended by osmosis.

Again, while most lipoid solvents do, as a matter of fact, cause hæmolysis, the absence of an effect on the part of pure olein, while a mere trace of an oleate is sufficient, shows that the solvent action exerted on the lipoids of the cell membrane is not the chief factor. A surface tension and adsorption effect is rather suggested, leading to modifications in the colloidal state of the membrane.

Mines (1912, p. 226) has shown that red blood corpuscles behave to the agglutinating action of trivalent ions as if coated with an emulsoid colloid. Now a suspension of lecithin in water behaves rather as a suspensoid towards electrolytes (Porges and Neubauer, 1907), being precipitated by bivalent ions in low concentrations. These facts suggest that the composition of the cell membrane is rather that of protein than of lecithin.

According to Pascucci (1905, p. 551), the stroma, or colourless portion, of the red blood corpuscles consists of protein, lecithin, cholesterol and a cerebroside. The greater part of this stroma forms the outer membrane. Various reasons are given for the belief that there is very little, if any, protoplasmic skeleton within the corpuscle, the chief reason being the separation, in certain conditions, of the whole of the hæmoglobin in large crystals within the corpuscle, while nothing is to be seen of any protoplasmic substance between the crystals.

The same investigator made artificial membranes of lecithin and cholesterol (p. 555) by impregnation of a fine silk tissue, tied over the end of a glass tube, with the fused lipoid or mixture of the two. Such membranes were found to be attacked by hæmolytic agents, saponin, cobra venom and tetanus toxin, in a similar way to blood corpuscles, becoming permeable to hæmoglobin. Lecithin was much more readily attacked than was cholesterol. Lipoid solvents attacked both, as would be expected, but dilute sulphuric acid had no action. On the other hand alkalies, both ammonium and sodium hydroxides, rendered them permeable. In this latter respect they differed from the normal cell membrane, which, as we have seen, is permeable to the former, impermeable to the latter.

The experiments of Garmus (1912) on the living skin glands of the frog lead him to the conclusion that the penetration of dyes into the cells of these glands has no relation to their solubility in lipoids, since some of those that obtain entrance are insoluble in lipoids. Moreover, poisons like saponin, sodium fluoride and other, which attack lipoids, do not affect the vital staining of the gland cells. It is possible, however, that secreting cells behave in a different way from the majority of other kinds of cells.

Peskind (1903, p. 420) comes to the conclusion, from experimental results which are not very convincing, that a "nucleo-protein" forms a constituent of the cell membrane, in conjunction with lipoids.

In respect of the question as to the penetration of substances into cells on account of their solubility in lipoids, a certain confusion is apt to be made in the interpretation of the action of such lipoid-soluble substances. It appears to be assumed sometimes that, if a particular substance, say chloroform, is more soluble in the lipoid membrane than it is in a watery liquid, the result will be that there is a greater concentration of the chloroform in the interior of the cell than in the surrounding liquid. On the contrary, if the solution inside the cell is the same as that outside, the concentration will be identical; the fact of greater solubility in the lipoid only means that the concentration in the *cell membrane itself* is higher. The meaning of the "partition coefficient" is that there is a particular ratio between the concentration of a substance in two phases, according to its relative solubility in them, so that, unless the interior of the cell has the same solvent power as the lipoid itself, the "partition coefficient" applies to the membrane only and not to the cell as a whole. Whether the concentration is higher in the cell protoplasm depends on the amount of lipoids which this contains. When it is found, for example, that narcotics as a class

are taken up by the nervous system in greater proportion than they are by other tissues, this must be understood to mean that lipoid constituents are in greater proportion in this tissue. It does not necessarily mean that the protoplasmic substance of the nerve cell itself is exposed to a greater concentration of narcotic than that of other cells is.

Experiments by Osterhout (1911) on Spirogyra show that the cells of this alga are permeable to both sodium chloride and to calcium chloride, as well as to many other salts, *when present alone*; the conclusion is drawn that the membrane is not lipoid, since these salts are insoluble in such substances. The remarkable fact was observed that a mixture of chlorides of sodium and calcium renders the membrane impermeable to both. These results are regarded as indicating a protein constitution. It appears to me, however, that caution must be exercised in interpreting them and that they indicate rather that a pure salt affects the membrane in such a way as to produce an abnormal permeability, which is of a temporary nature, since, in the experiments referred to, the cells were not permanently injured. From the fact that the natural cells were found to be isotonic with 0·375 molar sodium chloride, it must be concluded that they contain a considerable amount of osmotically-active crystalloids, which would diffuse out into the nearly pure water in which the cells normally live, unless the membrane were impermeable to salts.

That a simple protein membrane is insufficient to account for such impermeability is shown by the behaviour of some interesting protein membranes prepared by Newton Harvey (1912). When chloroform is shaken with solutions of egg-albumen, a membrane is formed on the surface of the drops by condensation of the protein in the manner described by Ramsden (1904). If these globules are allowed to stand in water, the chloroform diffuses out faster than water enters, so that they shrink; if lecithin be dissolved in the chloroform previously to the shaking with the egg-white, water is taken up sufficiently rapidly to prevent shrinking and, if left in an open vessel, the chloroform disappears entirely in the course of an hour or two and there remains, inside the delicate protein membranes, a colloidal solution of lecithin, partly in the form of granules which are visible under the microscope. When a dilute solution of neutral red is added to a suspension of these artificial cells in water, the lecithin granules take up the dye by adsorption and become red, so that an opportunity is given to test the permeability of the protein membrane as regards alkalies. It is found, contrary to the behaviour of the living cell, which is impermeable to sodium hydroxide but permeable to ammonium hydroxide, that the two alkalies pass through the protein membrane at an equal rate. The membrane of the living cell is therefore of quite a different composition from that which condenses on chloroform drops in a solution of egg-white.

Another instructive experiment made by the same observer is to take a solution of lecithin in benzene, instead of in chloroform, and to repeat the above procedures. The benzene of the droplets cannot, of course, diffuse away into water, but, if they be stained with neutral red and placed in ammonium hydroxide of 0·0001 molar concentration, the change to yellow is almost instantaneous, while even in 0·1 molar sodium hydroxide, it takes twenty minutes to produce the change. Ammonium hydroxide, in fact, is readily soluble in benzene-lecithin solution, while sodium hydroxide is not. But it is easy to show that wet benzene itself behaves in the same way.

Gelatine, stained with neutral red, is allowed to set in the bottom of an Erlenmeyer flask, which is then filled with water and inverted in a vessel of water. By means of a bent tube, benzene is passed up into the flask, where it forms a layer between the water and the gelatine. Various alkalies and acids can be added to the water in the flask by the same tube, and it will be found that benzene is permeable to ammonium hydroxide and to acetic acid, impermeable to sodium hydroxide and to hydrochloric acid, in fact it behaves like the cell membrane. According to these experiments, the cell membrane should be composed of benzene, which is absurd. Newton Harvey's experiment, in fact, tells us nothing as to the properties of lecithin when saturated with water; according to Pascucci, as we saw above, a lecithin membrane is attacked both by ammonium and sodium hydroxides. None of these experiments, indeed, affords proof that the cell membrane is composed only of lipoid material.

ACTION OF TOXIC SUBSTANCES

When cells are killed by various means, their semi-permeability is, as a rule, converted into complete permeability. But there are some agents which, when dilute, have not this effect, although they kill the cell. Formaldehyde in 4 per cent. solution destroys the semi-permeability, but in 0·2 per cent. solution, this property is preserved in an apparently normal state for a considerable time; so that, for example, Stewart (1901) was able to show that blood corpuscles, treated with dilute formaldehyde, retain their normal permeability for ammonium chloride and their normal impermeability for sodium chloride. Moreover, saponin and water cause the same change in permeability to ions that they do in living blood, although no laking takes place. It follows from these facts that the action of saponin or of water does not depend on liberation of hæmoglobin, but must be exerted on the cell membrane. When the corpuscles, after fixation by formaldehyde, are extracted with ether, which presumably removes the lipoids, the conductivity of the corpuscles is increased and saponin has no further effect in this direction. The inference seems to be that lipoid substances are an integral part of the membrane and that the action of saponin is on these substances, although the possibility is not to be forgotten that ether may produce other alterations in the nature of the membrane, apart from abstraction of lipoids.

A remarkable effect on blood corpuscles produced by cobra venom has been described by Noguchi (1905). Like snake poisons in general, this is hæmolytic in low concentrations, but different species of animals vary much in their sensitiveness to this effect. In great excess, cobra venom is not hæmolytic; on the contrary, it prevents the hæmolytic action of saponin. Even water, several times renewed, has no action on corpuscles subjected to the action of large quantities of cobra venom. It seems impossible to explain this fact except on the hypothesis that the membrane has become actually impermeable to water, as if converted into wax or india-rubber. When washed with sodium chloride, their normal behaviour to water is restored. It seems that some constituent of the membrane enters into combination with the poison, forming a substance which is insoluble in water, but decomposed by sodium chloride.

Ether and chloroform, like formaldehyde, have a different action in dilute and in concentrated solutions. In the latter, they increase permeability, in the former, they decrease it. Osterhout (1913) has shown this in the case of Laminaria by conductivity measurements. It is to be noted, however, that the decreased permeability is the reversible one and not associated with permanent injury to the cells, so that it seems to be the normal narcotic effect. Further facts will be found under the head of narcosis below.

A point to be remembered is that it is not to be assumed that, when a cell is killed, the semi-permeability of its membrane is necessarily lost. It may be fixed in some way.

A cell, dying naturally, may become surrounded by a tough impenetrable membrane. Penard (1890) made the following interesting observation. An Amœba, while living, had taken in the egg of a small worm. After the death of the Amœba, the egg hatched, but the worm was unable to escape through the surrounding membrane.

Heat, applied gradually, destroys the semi-permeability of the membrane. Even at 40° the pigment escapes from the red beet. A sudden rise of temperature to 100° appears to be a useful fixing method for certain histological purposes, but what its effect on the membrane may be, I am unable to state.

THE NATURE OF THE MEMBRANE

What conclusions may we, justifiably, draw from the various experimental data of the preceding pages?

In the first place, it seems certain that the membrane consists of substances in the colloidal state. The marked effect of electrolytes shows this, especially the fact that valency plays an important part.

The following observations of Szucs (1910) on the diminution of the permeability of Spirogyra to methyl violet are of interest. In order to produce a particular depth of staining in eight minutes, the concentrations required were of potassium nitrate, 0·08 molar; of calcium nitrate, 0·04 molar; of aluminium nitrate, 0·0005 molar. It will be seen that the effect is in relation to the valency of the cation, which probably acts in a coagulating manner

on the colloids of the cell membrane. Another important fact in this connection is that blood corpuscles are much more sensitive to saponin when suspended in isotonic sodium chloride than in isotonic cane-sugar, as found by Handovsky (1912, p. 413). For example, 0·002 per cent. saponin produced 98 per cent. hæmolysis in the former case, but only 20 per cent., under similar conditions, in the latter. The way in which this effect is produced is not quite clear. Although saponin may not be in colloidal solution in water, the experiments of Dumanski on molybdenum oxide, referred to on page 95 above, suggest that the presence of electrolytes may cause it to assume the necessary aggregated condition, and thus the electrolyte, also changing the sign of the charge on the corpuscles, may facilitate adsorption by electrical means.

In the second place, there are reasons, as we have seen, for rejecting the hypothesis of a membrane consisting of a simple kind of substances, lipoid or protein, alone, and for regarding it as a complex colloidal system of all cell constituents, together with those of the outer liquid, which diminish the surface energy at the interface.

Lepeschkin (1911), in fact, comes to the conclusion, as the result of an elaborate series of experiments, that a simple mosaic structure of lipoid and protein is not a satisfactory hypothesis, but that a colloidal complex is necessary. The effect of the addition of varying proportions of glycerol or castor oil to the collodion of which an artificial membrane is made will occur to the reader (page 95 above). The function of lipoids is suggested by Lillie (1912, 2, p. 17) to be that of increasing the stability of the other colloids, in fact as a protection from excessive aggregation, as described in the preceding Chapter (page 97). See Newton Harvey (1915).

Although lipoids must enter into the composition of the membrane, it seems evident that their relationship to substances which are supposed to be "lipoid-soluble is not that of solvents, in which case the laws of partition would be obeyed, but rather that of surface adsorption, owing to their state of colloidal dispersion. A colloidal solution of lecithin in benzene behaves quite differently from one of benzene in lecithin, that is, according to which is the external or continuous phase and which the internal or dispersed phase.

Ruhland (1913) gives strong evidence that, at all events as regards dyes and enzymes, permeability is not a question of solubility in the membrane, but of the dimensions of particles or molecules; that is, the membrane may be looked upon as a sieve. In this paper a full account of the literature on the subject is given.

An important point to remember is that the membrane must not be looked upon as an invariable permanent structure. Its permeability can be changed by reagents applied to the outside, as in the experiments of Osterhout, where sodium salts make it permeable to the Na ion, while the addition of calcium re-establishes the normal state of semi-permeability; other cases have been given above. Functional changes of the cell itself are also associated with changes in permeability, as will be shown in the next section. If, however, we look upon the cell membrane as an integral part of the protoplasmic system, as locally concentrated constituents of the cell, this behaviour will not seem so difficult to understand.

PHENOMENA IN WHICH CHANGES OF PERMEABILITY OCCUR

Supposing that the cell membrane becomes impermeable to substances to which it was previously permeable, what effects may be expected to follow? We know that, in a reversible reaction, the position of equilibrium depends on the relative concentration of the constituents of the system. Such a reaction will therefore continue to take place in one direction if the products are allowed to escape from the cell, but, if the membrane becomes impermeable to them, the reaction will come to an equilibrium and cease.

Take the case of starch or glycogen stored in a cell, which cell also contains an enzyme capable of causing their hydrolysis to sugar; if the membrane is impermeable to this sugar, the reaction soon comes to an end, partly on account of the back reaction, partly because the action of the enzyme is more or less paralysed by the accumulation of the products of its activity, as we shall see in Chapter X. But, as soon as the products are allowed to escape again, the reaction starts afresh. This consideration applies to any reversible reaction taking place in the cell.

From the powerful action of electrolytes on colloidal systems, such as that of

protoplasm, it will readily be understood how important are changes in the permeability of the membrane to these substances. That such changes occur is indicated, amongst other facts, by the experiments of M'Clendon (1912, 2), who found in *excited muscle* an increase of electrical conductivity, an index of increased permeability to ions, such as we have seen to happen in *Laminaria* under the influence of substances which increase the permeability of the cells.

It might be thought, perhaps, that the separation of electrolytes from an adsorbed state, owing to diminution of the active surface of the colloids in the cell by aggregation, as suggested by Macdonald (1909, p. 44), would account for this. But we know that the cell membrane is, under normal conditions, impermeable to ions, and acts as a non-conductor, so that increased production of ions inside the cell, apart from increased permeability of the membrane, would have no effect on the electrical conductivity of the tissue.

Lillie (1911), also, has brought forward evidence to show that all agents which cause increased permeability of the cell membrane act in an exciting manner. This is very noticeable in the case of the larva of *Arenicola*, which contains a yellow pigment to which the membrane is normally impermeable. When placed in pure sodium chloride, isotonic with sea water, the cells become tonically contracted, while at the same time pigment leaves them. This action of sodium chloride is prevented by calcium or magnesium ions, just as the increased permeability of *Laminaria* produced by sodium ions is prevented by calcium. Further discussion of the mechanism of muscular contraction will be found in Chapter XIII. One interesting consequence may be noted here. If the state of capability of being excited to contraction is connected with the semi-permeability of the membrane, it follows that when this state is changed into one of permeability the cell will be inexcitable as long as the state lasts; hence the "refractory period."

Pfeffer (1873) showed that, in the movements of the *sensitive plant*, water is pressed out from the cells of the pulvinus. A loss of turgor is thus caused, perhaps due to loss of semi-permeability and therefore of osmotic pressure. Blackman and Paine (1918) show that there is decrease in the osmotic concentration of the contents of the cell.

Narcosis.—There is a group of substances which act on living cells in such a way as to abolish temporarily those activities which we regard as manifestations of life. These are called "narcotics" or "anæsthetics." The former name means "making numb" or paralysing, while the latter obviously refers to abolition of conscious sensation, so that, in general use, the former is used to apply to the abolition of all forms of protoplasmic activity, including those of the nervous system, while the latter, strictly speaking, should refer only to consciousness. But, in point of fact, the substances themselves form one and the same group and the names are frequently used interchangeably.

As first pointed out by Hans Meyer (1899) and by Overton (1901), independently, the intensity of the narcotic action of a substance stands in relation to its partition coefficient between fats or lipoids and watery liquids; the more soluble it is in the former, the greater its effect. Now, although this fact shows how a narcotic obtains access to the interior of a cell, it does nothing more in explanation of its action than to suggest that it is in some way exerted on the boundary membrane. As pointed out above, the greater solubility in the membrane would only entail a greater degree of activity if this were due to some direct action on the membrane itself. The concentration in the water phase of the cell would not be increased by mere increase of solubility in the membrane alone.

What evidence, then, have we as to the action of narcotics on the permeability of this membrane?

As pointed out by Lillie (1912, 1), the property of rendering cells temporarily irresponsive to stimuli belongs to the most diverse classes of chemical compounds. The action of isotonic cane-sugar on muscle (see page 125 above) may be mentioned. At the same time, the particular group known as "anæsthetics," *par excellence*, such as ether, chloroform, alcohol, etc., are characterised by special activity of this kind, which seems undoubtedly to be connected with lipoid solubility. On the other hand, the mere fact of lipoid solubility does not make a substance an anæsthetic.

For example, capryl alcohol is a powerful anæsthetic, its "critical concentration" (Overton) being 0·0004 molar, compared with ethyl alcohol at 0·3 molar, that is, it is 750 times as powerful as ethyl alcohol. But benzene is a very poor anæsthetic, although its lipoid solubility is as great as that of capryl alcohol. The fact that benzene is only slightly soluble in water does not account for the fact, since, according to Rothmund (1907, p. 75), it is soluble in water to the extent of 1·4 parts in 2,000, while capryl alcohol is only soluble to the extent of 1 part in 2,000.

If lipoid solubility were the only condition making a particular substance a narcotic, it would be expected that the greater the lipoid content of an organ, the less would be the concentration of a certain narcotic required to produce its effect. Although this applies when we compare the central nervous system with other tissues, it has been shown by Choquard (1913) that it does not hold in the case of the heart muscle and skeletal muscle. The former, according to Erlandsen (1907), is considerably richer in lipoids than the latter and should therefore be more sensitive to all lipoid-soluble narcotics. Choquard's experiments show numerous exceptions.

We turn now to experiments with regard to the effect of anæsthetics on permeability. According to Lillie (1911), there is a general parallelism between the effect of agents in producing a state of excitation and their power of increasing the permeability of the cell membrane. If this is so, we should expect the opposite effect on the membrane to be produced by narcotics, which abolish the excitability. Lillie himself (1912, 2) has shown that the action of sodium chloride in causing excitation in the Arenicola larva, along with escape of pigment, is prevented by ether, alcohol, chloroform, and chloretone. He draws the conclusion that the characteristic effect of these substances is produced by an action on the cell membrane, making it more resistant to the action of substances which tend to increase its permeability. Although lipoid-soluble anæsthetics enter the cell immediately, the fact that magnesium chloride is a powerful anæsthetic, although it enters the cell with extreme slowness, indicates that the effect is essentially on the boundary membrane itself. Further evidence of the same nature is given in the experiments of Osterhout (1913) on *Laminaria*. In ether of 1 per cent., the electrical resistance of the cells *rises*, showing a decrease of permeability to salts, in 3 per cent., after a preliminary rise, the resistance *falls* and the tissue is killed. After exposure to 1 per cent. ether, recovery is complete; since recovery is a distinctive mark of anæsthetic action proper, it is reasonable to hold that it is the diminution of permeability which is associated with this effect.

As to the way in which the state of the lipoid constituents of the membrane is modified, we are as yet in the dark. A purely solvent action is precluded, since the lipoids would be washed away and the state be irreversible. This has been pointed out by Overton (1901, p. 51). There is no evidence that the state of colloidal dispersion of the lipoid is altered, that is as regards the *number* of particles. Lillie holds (1912, 1, p. 395) that the lipoid particles must increase in size by taking up the anæsthetic. In fact, Calugareanu (1910, p. 100) has seen this to occur in lecithin suspensions when ether or chloroform is added. It is evident that such a process would tend to decrease the interstices or pores of the membrane, if such a sieve-like structure be accepted, and that it would be reversible. According to Loewe (1913), narcotics change lipoids from lyophile into lyophobe colloids by surrounding them with an impermeable layer. Hence, such agents would diminish permeability so far as the water in the colloidal particles acted as a solvent, or carrier for solutes. No excitation would be possible in this state, because the membrane cannot be made permeable. It is found, in fact, experimentally, that narcotics lower permeability. Are the lipoid particles robbed of their water or not? The decrease of permeability indicates the latter, for otherwise they would shrink and allow more watery space for diffusion. On the other hand, the irreversible increase of permeability, leading to death, may well be due to actual dissolving away of the lipoids. The investigations of Czapek (1911) have shown that there is a close parallelism between the power of the various alcohols in killing cells and their power of lowering surface tension. As already mentioned (page 52), when the surface tension at the cell membrane is lowered to a certain degree, death results. This phenomenon is undoubtedly connected with the various degrees of lipoid solubility shown by the series of alcohols. It is difficult to say whether the surface tension as such plays any important part.

There is no doubt that adsorption of active substances, including narcotics, by the constituents of the cell membrane must play a considerable part, and indeed Straub (1912, p. 11) regards the adsorption theory as the most satisfactory one in respect to alkaloids.

An experiment by Calugareanu (1910, p. 101) shows that lecithin does not distribute itself between water and chloroform according to the usual rules of relative solubility. If chloroform be shaken up with an equal volume of a 0·5 per cent. watery lecithin "solution," instead of the lecithin being extracted by the chloroform, in which it is greatly more soluble than in water, what happens is that the chloroform layer only contains 8 per cent. of the total lipoid present, the rest is still present in the watery phase, but has taken up 50 per cent. of the chloroform. No doubt this behaviour is connected with the state of the lecithin as an emulsoid colloid, especially in presence of chloroform.

It is scarcely necessary to remark that, as yet, it is not possible to explain satisfactorily why changes of permeability should give rise to the various phenomena connected with the state of excitation or of narcosis; further investigation is required and it seems probable that a more intimate knowledge of the electrical conditions of the surface of the cell will give valuable information. We have seen (page 120) how the impermeability of the membrane to one only of the ions of a salt prevents the escape of the other, diffusible, ion, giving rise to a difference of potential between the two sides of the membrane, and how this can be changed by the presence of salts of which both ions are diffusible. But whether such changes in the polarisation of the membrane are sufficient to account for the change in permeability, as Lillie appears to hold, or whether the change in permeability is itself the primary factor, will come up for discussion later in Chapter XIII.

The effect of the substances to which Armstrong (1910) has applied the name "hormones" is clearly allied to the increase of permeability produced by fatal quantities of anæsthetics. These "hormones" are lipoid-soluble and coincide very closely with those substances which are known to abolish the semi-permeability of the membrane, such as ether, alcohol, toluene, etc. Their main obvious action is to set up an enzymic process which was previously in abeyance, such, for example, as the action of emulsin in the leaf of the cherry-laurel on a cyanogenetic glucoside also present. This is regarded by Armstrong as being due to an exciting action on the part of the "hormone" after entering the cell; but it seems to me that it falls better into line with other similar processes if it be looked upon as due essentially to the removal of some such obstacle as that of a membrane, which prevented the access of the enzyme to the glucoside.

Hæmolysis is of two kinds. One in which the surface membrane of the corpuscles is acted on by various hæmolytic agents, such as saponin, the other in which the corpuscle is broken up by osmotic swelling, as in the action of water. Substances acting on lipoids produce the first effect; hypotonic solutions, the second.

Ryvosh (1913) holds, with Hamburger, that, in hæmolysis by hypotonic solutions, the membrane is not destroyed, but merely stretched to such a degree that the pigment can escape. The ground for this view is, that, after treatment with water, or with 0·3 per cent. sodium chloride, although the relative volume of the deposit, after centrifuging, is 0·2 in the first case as against 0·8 in the second, yet, on placing in 2 per cent. sodium chloride and again centrifuging, the volumes became practically equal, 0·2 to 0·25. That is, although the corpuscles were greatly swollen in 0·3 per cent. sodium chloride, they could still contract under the influence of a hypertonic solution, showing that they retained their semi-permeability as regards sodium chloride.

Further details are beyond the space at our disposal and may be obtained from the general summary by Stewart (1909).

Secretion.—It is clear that constituents formed in gland cells must leave these cells by the side turned towards the lumen of the alveolus in connection with the duct. Apart from the actual chemical processes in connection with secretion, to be described in a subsequent chapter, changes in the permeability of the cell membranes must be taken into account. Various researches by Asher and his co-workers have brought out a number of facts, interesting in this connection. It is well known that atropine has the property of stopping the activity of secreting cells in general, and Garmus (1912) shows that, under the action of this alkaloid, the cells of the glands of the frog's skin take up less dye than normally. There is no reason to suppose that the actual stainable material is diminished, so that the result must be ascribed to a diminution of permeability, especially as pilocarpine, which excites the cells, has the opposite effect on staining. We saw previously that, in the excited muscle cell there is also an increase of permeability. It has been shown by Straub (1912, p. 22) that the action of atropine in antagonising that of muscarine on the heart is

due to the diminution of permeability towards muscarine, brought about by atropine.

The Nerve Synapse.—According to the view advocated by Sherrington (1906, p. 16) the communication of a nerve impulse to the cell body of another neurone takes place across a membrane, the "synaptic membrane." It is, therefore, owing to changes in the permeability of this membrane that impulses are allowed to pass or not. Whatever may be the actual chemical substance that diffuses through, or whether only a physical process is involved, there is every probability that the ions of dissociated salts play a large part in the transmission of nerve processes, so that it is a matter of importance to see what kind of action may be looked for.

We have as yet very little direct evidence on the question, but there are some observations which are of interest. Locke (1894) found that immersion of the sartorius muscle of the frog in 0·7 per cent. sodium chloride had the effect of preventing the muscle from contracting when the nerve to it was excited, although its direct excitability was not abolished and the effect was not produced if the nerve alone were immersed. Addition of traces of calcium salt to the solution restored the normal state. According to Overton (1904, p. 280), reflex excitability is lost in the absence of calcium from the central nervous system and one is obviously reminded of the action of calcium salts on colloids. Whether the synaptic membrane requires to be more or less semi-permeable, in the osmotic sense, in order to permit the excitatory process to pass, cannot be answered until we know more as to the nature of this process.

Certain facts to be described below with respect to reciprocal innervation in reflex action are made more explicable if we could imagine a membrane permeable to certain ions in one direction only. There is some evidence that the skin of the frog is permeable to sodium ions from without in, but not from within out. The most satisfactory evidence seems to be that the skin acts as a rectifier for alternating currents, that is, it allows the one part of the period, in which the current is flowing in one direction, to pass through more easily than that in which the current flows in the opposite direction (Bayliss, 1908, p. 235).

This result would also be obtained, as Höber justly points out (1911, p. 493), if the cell membrane on the inner side of the skin were permeable to one only of the ions of the salt. I found, in fact, that similar phenomena are shown by a system consisting of a solution of Congo-red inside a parchment paper membrane, which is permeable to the sodium ion of the salt only. Since a current can only pass continuously when a quantity of positive ions can pass to the negative pole equal to the negative ions passing to the positive pole, it follows that, if the positive pole is outside the membrane, which is impermeable to the negative ions, these can never get to the positive electrode outside at all; while, if this electrode is on the same side of the membrane as the anions, so that they can reach it without hindrance, the current will pass readily, because the cations can pass through the membrane.

It does not seem necessary, therefore, to assume an *irreciprocal permeability*, which is difficult to conceive. In any case, it would only be possible in the case of a living membrane, to which energy was being supplied by cell activity. Otherwise, there would be a spontaneous difference of potential kept up between the two sides of the membrane and the possibility of a perpetual motion machine.

Fertilisation of the Egg Cell.—In this process, it has been shown by M'Clendon (1910, p. 256) that the membrane becomes considerably more permeable to electrolytes, evidenced by the increase of electrical conductivity of a mass of eggs of the sea urchin on fertilisation. There is other evidence of increased permeability in the escape of pigment observed by Lillie, who regards the essential element in the artificial segmentation under the influence of certain salts as an increase in the permeability of the cell membrane (see also Lillie, 1917).

Gray (1913, 1916) found diminution of electrical resistance in Echinus eggs in the process of fertilisation, followed by return to or towards the normal.

The Permeability of the Blood Vessels.—It is plain that all substances necessary for the nutrition of cells and all those produced by the cells, so far as they pass into the blood stream, have to pass through the wall of the capillaries (except, perhaps, in the case of the liver—Schäfer, 1902). Some of these substances are in the colloidal state, and therefore, unless the cells are permeable to colloids, which does not seem probable, these colloids must escape between the cells, by a process like that of filtration. This question will come up for discussion later, but it may be remarked here that, as far as the blood proteins are concerned,

evidence already referred to (page 107 above) indicates that they do not serve for the nutrition of cells. On the other hand, the permeability of the capillary wall may let through proteins in pathological conditions. In dropsy the continuous flow of lymph, which is obtained from a canula in the subcutaneous tissue, contains protein and must have been filtered from the blood capillaries. Normally, the blood vessels are impermeable to colloids (see F. H. Scott, 1916).

PHENOMENA DUE TO ACTION ON THE CELL MEMBRANE ITSELF

There are many substances which exercise a powerful action on cell processes, but which can be proved in certain cases not to enter the cell at all, and in other cases, although they do enter the cell, they exercise no action after having obtained entrance.

One of these cases has been referred to in another connection, viz., the experiments of O. Warburg (1910, p. 313) on the action of alkalies on the oxidation processes in the developing egg of the sea urchin, in which it was found that the consumption of oxygen could be doubled by the addition of very small amounts of sodium hydroxide to the sea water in which the cells were immersed. Ammonium hydroxide, on the other hand, produced scarcely any effect. By previously staining the cells with neutral red, it could be shown that no sodium hydroxide entered the cell; whereas, if ammonium hydroxide was used, a rapid change of the dye to yellow showed that the alkali had entered the cell. The action of alkali on oxidation must, therefore, be exerted on the cell membrane itself.

The following observations of the same experimenter (1911, p. 425) are of interest in several ways. The young red blood corpuscles of the goose are distinguished by considerable consumption of oxygen. This process, unlike that of the sea urchin eggs, is not affected by salts. If, however, the cell membrane is destroyed by careful freezing and thawing, which does not affect the total consumption of oxygen, then the process becomes sensitive to salts, especially to barium chloride. The unavoidable conclusion is, that, as long as the membrane is intact, barium chloride cannot enter. In those cases in which it produces its effect on the intact cell, it must do so by intermediation of the membrane, since it cannot pass any further.

Newton Harvey (1911, p. 546), working on *Paramœcium*, found that the action of sodium hydroxide on the changes in behaviour, the formation of vesicles, cessation of movement, and final death were all produced without the entrance of the alkali into the cell substance. The same investigator later (1913), in a special series of experiments, showed that the method used was free from objection.

The experiments of Bethe (1909) on *Medusæ* showed that acids had an accelerating action on their movements, although no change of dye indicator within the cells occurred.

An experiment of Overton's (1904, p. 202) shows that the action of potassium on muscle is also on the surface only. A sartorius muscle is transferred from Ringer's solution, through 6 per cent. cane-sugar, to 2 per cent. potassium tartrate, in which no change of weight takes place, showing that the cells are completely impermeable to the salt, since the solution is isotonic with the cane-sugar. Nevertheless, the muscle is totally paralysed. On placing in Ringer's solution again, the excitability is quickly regained. This latter fact confirms the view taken of the action of the potassium salt as being on the cell membrane, since, if it had penetrated into the interior, it is difficult to understand how it could pass out again with such rapidity.

Overton also showed (1902, 2), as will be remembered, that, if all the sodium chloride be washed out of a muscle, it becomes inexcitable until more sodium chloride is supplied. Now Fahr (1909) states that the only satisfactory explanation of the results of his experiments is that the muscle cells themselves normally contain no sodium at all. But since sodium is necessary for their activity, it follows that it must act on the membrane, as this is the only part of the cell with which it comes into relation.

Conclusions of the same kind are drawn by Straub (1912, p. 14) with regard to the action of calcium on the heart muscle, which is impermeable to this substance. Fig. 46 illustrates this point. In the tracing, the first three beats are under the action of Ringer's solution containing sodium, potassium, and calcium. At the signal, the solution is suddenly changed for a similar one without calcium. It is seen how the want of calcium is shown in the first beat succeeding the change. At the end of the signal, the normal Ringer's solution is replaced, with its action on the very next beat. Straub reckons that the effect must be manifested in less than 0·1 second. It is to be remembered that the interior of the cells contain calcium, which could not diffuse out by the time at which the action of a calcium-free solution is manifest. The calcium could merely be removed from the outer surface of the cells.

The experiments of the same investigator on the effect of muscarine on the heart of *Aplysia* (1907) have already been described. When this organ is allowed to lie in a small quantity of a solution of the drug, it is noticed that the effects of the poison are only seen while there is a particular concentration of it left in the solution; the heart then recovers, although it is found that muscarine has been stored inside its cells and remains there. It is plain that the effect was only to be seen while the poison was in the act of passing through the membrane, so that it must be supposed that it leaves the membrane, and passes into the cell substance as soon as there is less than a certain minimal concentration in the outer liquid.

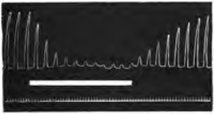

FIG. 46. EFFECT ON THE FROG'S HEART OF DEPRIVATION OF CALCIUM.—The first three beats are normal in Ringer's solution (which contains calcium). At the beginning of the white space, this solution is suddenly changed for one otherwise similar, but from which the calcium has been omitted. The effect is shown at once on the first beat after the change. At the end of the white space, the normal solution is replaced, with an immediate effect. Time in seconds. Note that less than 0·1 second is required to affect the muscle cells, so that the action must be exerted on the colloidal system of the cell membrane. This is the only part of the cell which could be deprived of calcium with such rapidity.

(Straub.)

Somewhat similar results were obtained by Neukirch (1912) with the action of pilocarpine on the excised small intestine of the rabbit, immersed in warm oxygenated saline solution. The addition of pilocarpine to this solution causes a great increase of tonus, which slowly decreases but does not disappear, even in several hours. But, if the pilocarpine solution be changed for fresh saline, a second increase of tonus occurs as the alkaloid diffuses out from the cells into the saline. A remarkable fact is that if, as soon as this tonus has developed, the pure saline be exchanged for the original pilocarpine saline, so that diffusion ceases on account of equality of concentration inside and outside of the cells, the tonus also disappears. It seems, then, that in this case, the actual presence of

equally concentrated solutions of the alkaloid on both sides of the membrane is of no effect or a minimal one. The characteristic effect is manifested only while the drug is in the act of passing through the membrane.

As long as we remember that the cell membrane is a modifiable part of the cell system, the various facts described above need not cause surprise.

PERMEABILITY TO SOLIDS

The phenomena of phagocytosis, and of digestion in protozoa generally, show that solid particles are able to enter the cell. In secretion, again, we find that solid products sometimes leave the cell. This takes place, in all probability, by a process similar to that by which a needle can be dropped through a soap film without breaking the film, which completes itself over the end of the needle as it passes through. The facts are not incomprehensible on the hypothesis that the cell membrane is a local concentration due to the action of surface forces, although it would be so if the membrane were a fixed solid layer. In the latter case, a hole would be formed if a solid entered the cell.

SUMMARY

Since protoplasm has the properties of a liquid and can also be shown to contain free, uncombined salts, there must be some means by which free diffusion between the contents of a cell or organism and the surrounding medium is controlled.

There is every reason to suppose that the regulation of the passage of substances between the inside and outside of a cell is effected by means of a film or membrane.

The membrane of the cell must allow water to pass freely, but hold back dissolved substances. Such a membrane is known as a semi-permeable one.

Artificial membranes can be made of various degrees of permeability; thus, some will only hold back colloids, others will allow certain crystalloids to pass, but not sugar, and so on.

Different views are held as to that property of a membrane which makes it permeable to some solutes and impermeable to others. Reasons are given in the text for accepting, with some modifications, the original sieve theory of Traube, according to which the passage of a solute through a particular membrane, depends on the size of the pores in the membrane in relation to the molecular, or particulate, dimensions of the solute. The hydration of solutes must be taken into account. In a few cases, the question of solubility in the substance of the membrane appears to play a part.

The protoplasmic substance of the cell is capable of forming a new membrane on a fresh surface. The substances present in the protoplasm which lower surface energy, and there are a large number of them, will be concentrated at the interface between protoplasm and external phase, and some of them may be coagulated. In this way a membrane is formed. It is to be noted that the cell membrane is, accordingly, an integral part of the cell system, and capable of modification with changes in the composition of the cell contents.

In this way a difficulty is overcome. If the cells are always impermeable to such solutes as sugar, amino-acids, and salts, how is growth to take place or the functions of the cell to be performed? We must conclude that the permeability of the membrane is not always the same; a fact which is also demonstrated by experiment.

The difficulty alluded to has caused certain investigators to deny altogether the existence of a semi-permeable membrane covering the cell protoplasm. Evidence of various kinds is given in the text, which shows that, in the condition in which cells are usually met with, they are actually impermeable to crystalloids.

This evidence consists in the permanent change of volume which cells undergo

under the action of various dissolved crystalloids, in the difference between the concentration and nature of crystalloids in the interior of the cell and in the outer medium; and, lastly, in the resistance opposed by living cells to the passage of electrical currents, notwithstanding the fact that they contain free electrolytes.

Although this may be regarded as the usual state of cells at rest, their permeability may be altered, without killing them, and therefore reversibly, by the action of various substances on the membrane. Of these we may mention electrolytes in particular. Narcotics and light are also found to have an influence.

The chemical nature of the membrane depends on the constituents of the protoplasm which lower surface energy. As fatty or lipoid substances possess this power in a marked degree, it is to be expected that the membrane will manifest many of the properties of lipoids. At the same time, reasons are given for not accepting the view that the cell membrane consists of lipoids alone, and still less that it consists of protein alone. The various substances of which it is composed exist in a complex colloidal intermixture in a more intimate connection than a mere mosaic of lipoid and protein.

It appears that, as a general rule, it may be stated that the cell membrane is *always* permeable to substances soluble in lipoids, but whether this fact is essentially due to the solubility itself, or to some other property, such as surface tension or molecular dimensions, is uncertain. The apparent solubility of many dyes and other substances in solutions of lipoids is not a true solution, but a surface adsorption on the colloidal particles of the lipoid. These dyes are insoluble in the lipoid itself. As regards substances insoluble in lipoids, the permeability of the membrane is capable of variation, so that, while being usually impermeable to salts, sugar, etc., it may sometimes become permeable to them. This latter fact necessitates a complex structure.

Various instances are given in the text which show that changes of permeability do actually take place in functional processes. The state of excitation of muscle, narcosis, secretion, the passage of the nerve impulse from one neurone to another or to a muscle cell, the fertilisation of the ovum and changes in the walls of the blood vessels are referred to.

Certain cases are known where substances produce profound changes in cell processes without passing beyond the membrane. The action of alkali on the oxidation process of sea urchin eggs and on the movements of medusæ, and that of potassium, sodium, and calcium ions on muscle are of such a kind. In other cases, the substance, muscarine or pilocarpine, only produces its effect during its passage through the membrane.

In brief, the cell membrane is a local concentration of constituents of the cell protoplasm due to their property of lowering surface energy of some kind. Substances present in the external medium, if possessing the same property, may also take part. The properties of the membrane are, therefore, not fixed, but capable of modification according to the chemical processes taking place in the cell, or they may be changed by influences on the outside. It is to be regarded as a part of what we may, for the present, call the "living system" of the cell. In its resting state, as usually investigated, it is impermeable both to colloids and to the majority of crystalloids, but may become, temporarily, permeable to all crystalloids and perhaps to some colloids.

LITERATURE

Höber (1911), Chapters VI., VII., and XIII. Overton (1907). Zangger (1908).

Properties of Lipoids.
Maclean (1918).

CHAPTER VI

OSMOTIC PRESSURE

THE fact that the metal palladium allows hydrogen to pass freely through it, while refusing such passage to nitrogen, enabled Ramsay (1894, p. 206) to make an interesting experiment. A vessel of palladium was filled with nitrogen and connected to a mercury manometer. It was then immersed in an atmosphere of hydrogen and the mercury was seen to rise steadily in the manometer. Why did this nappen?

The reason is that hydrogen passes through the walls of the vessel until its concentration or pressure becomes equal within and without; but, as the nitrogen cannot escape to give room for the hydrogen which enters, the amount of gas inside the closed vessel must increase and the total pressure rise.

The fact can also be shown by the use of a membrane of water, or, rather, a parchment-paper membrane soaked in water. Such a membrane is freely permeable to carbon dioxide, because the gas is soluble in water, but almost impermeable to oxygen and nitrogen. If, therefore, we take a bell-shaped vessel, and tie over the large end a wet parchment-paper membrane, connect the interior to a manometer, and then immerse the vessel in carbon dioxide, the pressure will rise rapidly inside for similar reasons as in the case of hydrogen and palladium.

Now we know, by what is usually known as *Dalton's Law*, that, in a mixture of gases at a certain pressure, this pressure is divided between the different gases in proportion to their relative volumes, or, in other words, the total pressure of a mixture of gases is equal to the sum of the pressures which each alone would exercise if it alone filled the vessel. Suppose that we have a mixture of nitrogen and carbon dioxide consisting of one-fifth nitrogen and four-fifths carbon dioxide at atmospheric pressure. The partial pressure of the nitrogen is one-fifth of 760 mm., that is, 152 mm. of mercury; this is also called its "tension." If such a mixture is put in a vessel as described above and pure carbon dioxide placed on the outer side of the membrane, the pressure will rise by carbon dioxide passing in until its tension is equal on both sides. But, since gases are compressible, the relative volume of the nitrogen will have been diminished by the process, so that it is better for the sake of description to imagine that, before immersion in the carbon dioxide atmosphere, we have raised the internal pressure by forcing in more of the gaseous mixture until the manometer reads 152 mm., that is, until the pressure is increased by the tension of the nitrogen while that of the carbon dioxide is that of the atmosphere. By this means we avoid the further inflow of carbon dioxide, and we find that the gauge remains stationary at 152 mm. of mercury, if the barometer stands at 760 mm. We have thus a measurement of the tension of nitrogen in the mixture. Of course, the pressure will not remain indefinitely at this point, since nitrogen is not absolutely insoluble in water, and it will therefore pass very slowly through the membrane, until the composition of the mixture is the same on both sides and no pressure will be shown on the manometer.

Let us now take an analogous experiment with a liquid system. We have seen in the preceding chapter that a membrane of copper ferrocyanide is freely permeable to water, while refusing passage to cane-sugar in solution in water. Pfeffer (1877) made a number of experiments in which the membrane was supported in the pores of a clay cell in order that it might be able to withstand the pressures developed. He found that these pressures, spoken of in the case of

solutions as "osmotic pressures," were directly proportional to the concentration of the solute and to the absolute temperature.

The process by which water passes through a membrane from a solution on the one side to another solution on the opposite side had been known, since the time of Dutrochet (1827, p. 393), as "endosmosis" or "exosmosis," so that the pressure due to this passage of water was naturally called "*osmotic.*"

The experiments made by Pfeffer have served as the starting point of subsequent work on osmotic pressure, especially as the basis of the important theory of solutions put forward by van't Hoff; I have therefore given his portrait in Fig. 47.

The similarity of the process in gases and in solutions is obvious, but the relationship was not made clear until van't Hoff (1885, 1) was led by thermodynamic considerations (see Cohen's book, 1912, p. 225) to the view that the pressure developed by a substance in solution is identical with that which it would exert if converted into gas of the same volume and temperature; in other words, the solute behaves as if it were in the dispersed molecular condition of a gas and the solvent were absent.

This statement does not necessarily imply that the state of the solute is actually that of a gas, although, as we shall see later, a kinetic theory, similar to that of gases, gives, on the whole, the most satisfactory explanation of the phenomena. It should be kept in mind that the facts of osmotic pressure, their connection with vapour pressure and so on, are independent of any theory of their origin.

W Pfeffer

FIG. 47. PORTRAIT OF PFEFFER.

Signature from Charter Book of the Royal Society.

On account of the importance of van't Hoff's theory, the actual words of the author himself (1885, 2, pp. 42 and 43) may be given:—

1. "*Loi de Boyle pour les Solutions.*—La pression osmotique est proportionelle à la concentration, si la température reste invariable.

2. "*Loi de Gay-Lussac pour les Solutions.*—La pression osmotique est proportionelle à la température absolue, si la concentration reste invariable.

Ce sont là les analogies qui ont été démontrées et verifiées en détail dans le travail cité (the preceding paper, 1885, 1); elles ont rapport à la variation de la pression avec les circonstances. Je vais ajouter maintenant une troisième

proposition, ayant rapport à la grandeur absolue de cette pression, et n'étant, en réalité, autre chose qu'une extension de la loi d'Avogadro.

3. "*Loi d'Avogadro pour les Solutions.*—La pression exercée par les gaz à une température déterminée, si un même nombre de molecules en occupe un volume donné, est égale à la pression osmotique qu'exerce dans les mêmes circonstances la grande majorité des corps, dissous dans les liquides quelconques."

At normal temperature and pressure one gram-molecule of a gas occupies a volume of 22·4 litres, so that if one gram-molecule of a solid be dissolved in 22·4 litres of water, its osmotic pressure should be one atmosphere, as may also be seen from the following consideration. To compress one gram-molecule of a gas to the volume of one litre, which is the volume occupied by any solute in what is known as molar concentration, requires, by Boyle's law, a pressure of 22·4 atmospheres.

Let us take an example from one of Pfeffer's experiments. A 4 per cent. solution of cane-sugar gave at 15° an osmotic pressure of 208·2 cm. of mercury. By Gay-Lussac's law, supposing it to apply, this would be, at 0°, $208 \cdot 2 \times \frac{273}{273+15} = 197 \cdot 4$ cm. mercury. One gram-molecule of the sugar weighs 342 g., so that the number of litres of a 4 per cent. solution required to contain 1 gram-molecule is $\frac{342}{40} = 8 \cdot 55$. Hence its osmotic pressure should be $76 \times \frac{22 \cdot 4}{8 \cdot 55} = 199$ cm. mercury, a very close agreement, considering the difficulty of the measurement.

This example will serve to show the justification of van't Hoff's point of view. The experiments of De Vries on isotonic solutions, referred to in the preceding chapter, gave further confirmation of its correctness.

Before proceeding further, we must insist on the fact that the theory was only intended to apply to *dilute* solutions. For the present purpose we may define dilute solutions as being those in which the number of molecules of the solute is so small in proportion to those of the solvent that any effects due to the mutual action of the molecules of the solute, to their actual volume, or to combination with the solvent, in the sense of hydration or solvation, may be neglected.

When we come to *concentrated* solutions, these factors have to be taken into account, as van't Hoff himself (see Cohen's book, 1912, p. 282) pointed out with reference to the treatment of the question from the kinetic point of view. In fact, the osmotic pressures of such solutions are found to be higher than the simple gas law would lead us to expect, the deviations becoming greater as the concentration rises.

The most important work on concentrated solutions is that done by Morse and his collaborators in the United States (1901, etc., summary in 1914) and by Berkeley and Hartley in England (1906, 1). These experiments were made on solutions of cane-sugar. A further series of measurements on calcium ferrocyanide was made in 1908 by Berkeley, Hartley, and Burton. As to the interesting methods employed by these observers, the reader is referred to the monograph by Morse (1914) and that by Findlay (1913). The preparation of the copper ferrocyanide membrane is of especial importance.

In the endeavour to find a formula which applies to concentrated solutions, as well as to dilute ones, it is obvious that, by the introduction of a sufficient number of empirical constants, this would not be difficult. On the other hand, if a physical meaning can be given to the constants introduced, although it may not, for the present, be possible to determine them by an independent method, such an expression is to be preferred. For this reason, in the following pages, I have adopted the point of view of van der Waals (1873) and, as regards details, that of Otto Stern (1913). This treatment consists in the application of the *van der Waals' equation of state* to solutions, and it must not be supposed that no other point of view is possible. The point of view of the doctrine of energy, or thermodynamics, for example, as given by Findlay (1913), leads to a logarithmic formula and affords results which are, of course, cogent if based on correct foundations, but it does not seem to me to help us far in understanding the factors at work. Nernst (1911, p. 155) appears to be of the same opinion. It is pointed out by Arrhenius (1912, p. 6) in reference to the selection by van't Hoff of the thermodynamic method, that, at that time, the kinetic theory was not so manageable as the former. Boltzmann, however, brought the kinetic theory into favour again by reducing it to an application of the theory of probabilities. The application of the kinetic theory to liquids will be found discussed in Nernst's book (1911, pp. 212-219).

The simple general gas law

$$PV = RT$$

is not, in reality, of universal application even to gases, and fails especially under high compression. It gives more accurate results the higher the temperature, a fact which is significant in connection with the data obtained by Morse and his co-workers (1912, p. 29). The osmotic pressure of a molar solution (weight normal, see below) at 5° was found to be 1·115 times that calculated; at 40° it was only 1·085 times, and at 80° the values agreed.

The failure of the simple Boyle-Gay-Lussac law to express the behaviour of gases at any temperature and pressure led *Van der Waals* (1873, *see* Bibliography) to consider the causes of the failure, and to formulate a more general law, which is usually stated thus:—

$$\left(P + \frac{a}{V^2}\right)(V - b) = RT.$$

We notice that P is increased by a new factor, which is a function of V, while V itself is diminished by another factor, b.

We will first consider this latter quantity, which has to do with the actual volume taken up by the molecules themselves. If molecules have a real concrete existence, and all recent work shows that they have, they must occupy space. The concordance between the values of Avogadro's constant, obtained by various methods as referred to in Chapter IV. above, is, in itself, sufficient proof of the actual existence of molecules. In gases at ordinary temperatures and pressures, the volume taken up by the molecules themselves is negligible in comparison with the space in which they are free to move. Larmor (1908) has pointed out that, if we imagine the molecule of a gas at atmospheric pressure to be magnified so that it has a diameter of 1 cm., there will only be one molecule in two litres; or the space taken up by the actual molecules themselves is only about one four-thousandth part of the total volume of the gas. When the gas is compressed, the volume of the molecules is not diminished, so that the relative fraction of the volume taken up by them becomes more and more pronounced. V, therefore, in the simple gas equation, that is, the space free for the molecules to move in, is actually the volume as measured, diminished by the space occupied by the molecules. This space is not necessarily the size of the chemical molecules themselves, but the distance at which they begin to resist being pressed closer together, and is, according to van der Waals, four times the former quantity in the rarefied state. It diminishes to about half this value as the total volume of the gas decreases under pressure.

Turning to liquids, and remembering that van der Waals applies his formula to pure liquids, non-associated, that is, consisting of single molecules, we may, as a first approximation, expect that, if we reckon the concentration of our cane-sugar solution as being the number of grams dissolved in 100 c.c. of water, so that a 10 per cent. solution is made by adding 10 g. of sugar to 100 c.c. of water, instead of taking a solution containing 10 g. of sugar in 100 c.c. of solution, better correspondence of osmotic pressure measurements with the theoretical ones would be obtained. This is in fact the case, as the following numbers from the experiments of Morse and Fraser show:—

Concentration.		Osmotic Pressure in Atmospheres.		
			Calculated.	
As Weight-Normal.	As Volume-Normal.	Observed.	From Volume-Normal.	From Weight-Normal.
0·1	0·098	2·59	2·34	2·39
0·3	0·282	7·61	6·74	7·17
0·5	0·452	12·75	10·81	11·95
0·8	0·684	20·91	16·36	19·12
1·0	0·825	26·64	19·73	23·90

By taking, in this way, what are called weight-normal instead of volume-normal

solutions, we are allowing for the value of the molecules of the solute, or taking $V - b$ instead of V in the simple equation.

But this procedure, as the table shows, is not a complete solution of the question, and we must also take into consideration the remaining constant of van der Waals, viz., a, which refers to the mutual attraction of the molecules, and therefore acts in the opposite way to b. This mutual attraction of the molecules has been already met with in Chapter III., in the case of liquids, as the internal pressure of Laplace, giving rise to the surface tension. These attractive forces are naturally less the further the molecules are from one another. They are in fact inversely proportional to the square of the volume occupied by a given number of molecules, *i.e.*, $\frac{a}{V^2}$. We must then increase P, in the simple gas equation, by this quantity.

In the *application of the van der Waals theory to solutions* I propose to follow, in the main, the treatment of Otto Stern (1912), since it is, on the ~~whole, capable~~ of easier explanation than the similar one of Berkeley (1907). For a complete account, however, the original papers must be consulted.

In the first place, we must not expect even dilute solutions to obey the simple gas law exactly, because the solvent itself is, as regards its molecular state, very concentrated when compared with a gas. In other words, its molecules are closely packed. According to van der Waals, at the boiling point, the volume of the molecules is about one-quarter of the entire space occupied by the liquid.

That there is space between the molecules of a liquid is shown by the fact, amongst others, that liquids are not altogether incompressible. Parsons and Cook (1911, p. 343) find that water at 4° can be compressed to 87 per cent. of its volume by a pressure of 4,500 atmospheres, and ether at 35° to 80 per cent. of its volume by 4,000 atmospheres.

Moreover, the molecules of the solvent affect those of the solute in both the attractive and the repulsive ways of the van der Waals equation; so that it is, in point of fact, rather unexpected to find, even in the case of dilute solutions, that the osmotic pressure is so nearly equivalent to the gas pressure of the solute. The reason for this, according to Stern, is the presence of the semi-permeable membrane itself, which causes the effects due to both the attractive and repulsive forces to be compensated in dilute solutions in the following way:—As regards a, a molecule of the solute which hits against the membrane is surrounded on *all* sides by the solvent, since the membrane is permeable to these. The attractive forces are therefore equal on all sides, as if the membrane were not present, and play no part in the production of the osmotic pressure, which can only be affected by forces which are unequal on the two sides of the membrane. As regards b, an increased osmotic pressure must undoubtedly be caused thereby, but a part of the total osmotic pressure, and, in fact, a part which is exactly equal to that due to the volume of the molecules, is taken up, not by the membrane, but by the molecules of the solvent in the act of passing through the membrane. A certain part of the membrane is occupied by molecules of the solvent, instead of membrane substance, so that a certain number of the molecules of the solute hit against these molecules of the solvent, instead of against the membrane, and are therefore inactive osmotically. This point of view is developed in a somewhat different manner by Haldane (1919).

Additional considerations must be taken into account when concentrated solutions are concerned. In dilute solutions, the "molar fraction," that is, the number of molecules of the solute in proportion to those of the solvent, is so small that mutual action may be disregarded. This is not the case with concentrated solutions, and Otto Stern has developed the following modification of the van der Waals formula:

$$\left[\pi + \frac{a_1 - a_{1\cdot2}(x_0 - x)}{v^2}\right] \times \left[V - b_1 + b_{1\cdot2}(x_0 - x)\right] = RT,$$

where π is the osmotic pressure, a_1 and b_1 are the van der Waals constants of the pure solute, $a_{1\cdot2}$ and $b_{1\cdot2}$ are constants depending on the attraction and repulsion respectively between the molecules of solvent and solute, and $x_0 - x$

is the difference between the concentration of the solvent outside the membrane and in the solution itself.

We note that a of the van der Waals equation is diminished by a factor expressing the attraction between the molecules of the solute and those of the solvent, which acts in the opposite direction as regards osmotic pressure to that between the molecules of the solute itself. The attraction between the molecules of the solvent and solute pulls the molecules of the solute away from each other, in opposition to their mutual attraction. The necessity for the introduction of $x_0 - x$ is that the concentration of the solvent inside the membrane is less than that outside by the space taken by the molecules of the solute. For similar reasons, the repulsive forces expressed by b are less than in the simpler case of a pure liquid.

The whole process of derivation of the formula is beyond the limits of this book, but there are one or two points to be noted in connection with it.

Owing to the fact of its containing two additional constants, it is not to be wondered at that it can be made to satisfy experimental results. These new constants, unfortunately, cannot as yet be tested experimentally by an independent method, but, at the same time, it is a matter of some satisfaction to possess an equation, similar in form to that of van der Waals, containing only factors to which a physical meaning can be assigned.

If the solvent is an associated liquid, like water, the equation still applies, although, of course, the numerical values of the constauts will not be the same.

Consider further that the two van der Waals constants have opposed to them other constants by which their value is reduced, and it will be obvious that in solution a substance should obey the ideal gas law more closely than in the gaseous state. Suppose that we are dealing with two easily miscible substances whose critical points are not very far removed from one another, so that their molecular state may be considered to be similar, then $a_{1\cdot2}$ and $b_{1\cdot2}$ are of the same order as a_1 and b_1. Moreover, the difference between the concentrations of the pure solvent itself and that which it has in the solution is nearly identical with the concentration of the solute, or $\frac{x_0 - x}{v}$ is very nearly equal to $\frac{1}{v}$, which is the concentration of the solute; $x_0 - x$ is, therefore, practically unity. This being so, the factors representing a will nearly cancel out, as will also those representing b, and a gas in solution will obey the ideal gas law more closely than it does in the gaseous state.

This remarkable result was tested by Otto Stern in the case of solutions of carbon dioxide in methyl and ethyl alcohols, acetone, and methyl and ethyl acetates, at low temperatures in order to avoid high pressures. The values actually measured were the absorption coefficients, and from these the osmotic pressures were calculated by a formula due to Nernst, taking account of the increase of the coefficient as the pressure increased.

The following numbers were obtained in the case of methyl alcohol at $-78°$ C., and will serve as an illustration. The column headed "Theoretical osmotic pressure" gives the values calculated from the simple gas equation, and it will be noticed how closely the observed values correspond to these, deviating only at the higher pressures. The last column gives the corresponding pressures in the gaseous state, as calculated by the van der Waals formula.

Pressure in Mm. Hg.	Concentration in Mols. per Litre.	Theoretical Osmotic Pressure.	Observed Osmotic Pressure.	Gas Pressure by van der Waals' Formula.
50	0·49	7·93	7·93	7·05
100	0·98	15·7	15·7	12·1
200	1·97	31·6	31·6	18·1
400	4·02	64·3	63·1	10·8
700	7·30	116·8	113·2	– 44

THE CAUSE OF OSMOTIC PRESSURE

The basis of the foregoing considerations has been that of the kinetic theory, according to which the osmotic pressure, developed by a solution constrained by a membrane permeable only to the solvent, is due to the impacts of the molecules of the solute against the membrane through which they cannot pass (Nernst, 1911, p. 244). It is well to note that there are other views on the question, such as surface tension, attraction of solute for solvent, and so on, but it would exceed the scope of the present book to discuss them. Although van't Hoff made use of the thermodynamic method in the quantitative mathematical treatment of osmotic pressure, he interprets the phenomenon in terms of the kinetic theory as given above (see p. 482 of his paper, 1887). For our purposes, the kinetic theory satisfies requirements best. Those who are interested in the question are referred to the monograph by Findlay (1913, pp. 65-76), and to the paper by Callendar (1908). Callendar remarks, "It is probable that all the theories possess some elements of truth, and that they may be to some extent merely different aspects of the same phenomenon."

HYDRATION OF SOLUTE

There is one point that requires a few words. Many solutes are hydrated in solution in water. That is, each molecule is associated with a larger or smaller number of water molecules. The result of this is that the number of molecules of water in a given volume is reduced, although that of the solute is not.

As far as dilute solutions are concerned, as Nernst (1911, pp. 271 and 469) points out, this fact will have no influence on the osmotic pressure, however measured. The number of molecules of water is so great in proportion to those of the solute that the fixation of a certain number of them will have no measurable effect. On the other hand, calculations of the osmotic pressure of concentrated solutions of cane-sugar, made on the hypothesis that each molecule is associated with five molecules of water, gives values more nearly approximating to those obtained experimentally (see Findlay, 1913, p. 42).

There is at present much difference of opinion as to the nature of this hydration. For example, it is stated by Callendar (1908, p. 498) that the conclusions of Jones and Bassett (1905) are "diametrically opposed" to his.

METHODS OF MEASUREMENT

The *direct* measurement of osmotic pressure, either by Pfeffer's method of measuring the pressure produced in the osmometer when one side of the membrane is immersed in water at atmospheric pressure, or by that of Berkeley by measuring the pressure necessary to be applied to the solution in order to prevent passage of solvent in either direction, is of considerable experimental difficulty, and only applicable in certain cases, owing to the fact that we know of so few appropriate semi-permeable membranes. In practice, the determination of other properties, which are related in a known way to the osmotic pressure, is usually resorted to. Fig. 48 shows the construction of some of the cells used by Morse.

Before passing to the indirect methods, a direct method due to Fouard (1911) will, perhaps, sometimes be found useful. In speaking of the semi-permeable membranes prepared by Traube, that made by the action of tannin on gelatine was referred to. Fouard makes use of this, but instead of measuring the pressure in a manometer, he balances it by the use of solutions of cane-sugar of known osmotic pressure outside. It is clear that the approximate osmotic pressure of the solution inside should be known, in order to save a large number of preliminary trials. A small cylinder of silver gauze is taken, immersed in 6 per cent. collodion in order to form a film, washed with water, and then filled with 1 per cent. gelatine, which is then poured out. After soaking for five to six days in dilute tannin solution, it is ready for use. It should be kept in dilute solutions of the membrane formers, presumably gelatine inside and tannin outside, or vice versa. For use, it is connected with a capillary tube, bent horizontally so as to be at the same level as the top of the outer solution. The solution whose osmotic pressure is to be measured is placed inside, so as to form a meniscus in the capillary tube. If the osmotic pressure of the cane-sugar solution is greater than that of the inner solution, water will pass out and the meniscus will move towards the cell and vice versa. By adding either water or sugar, as the case may be, a solution can be found which has the same

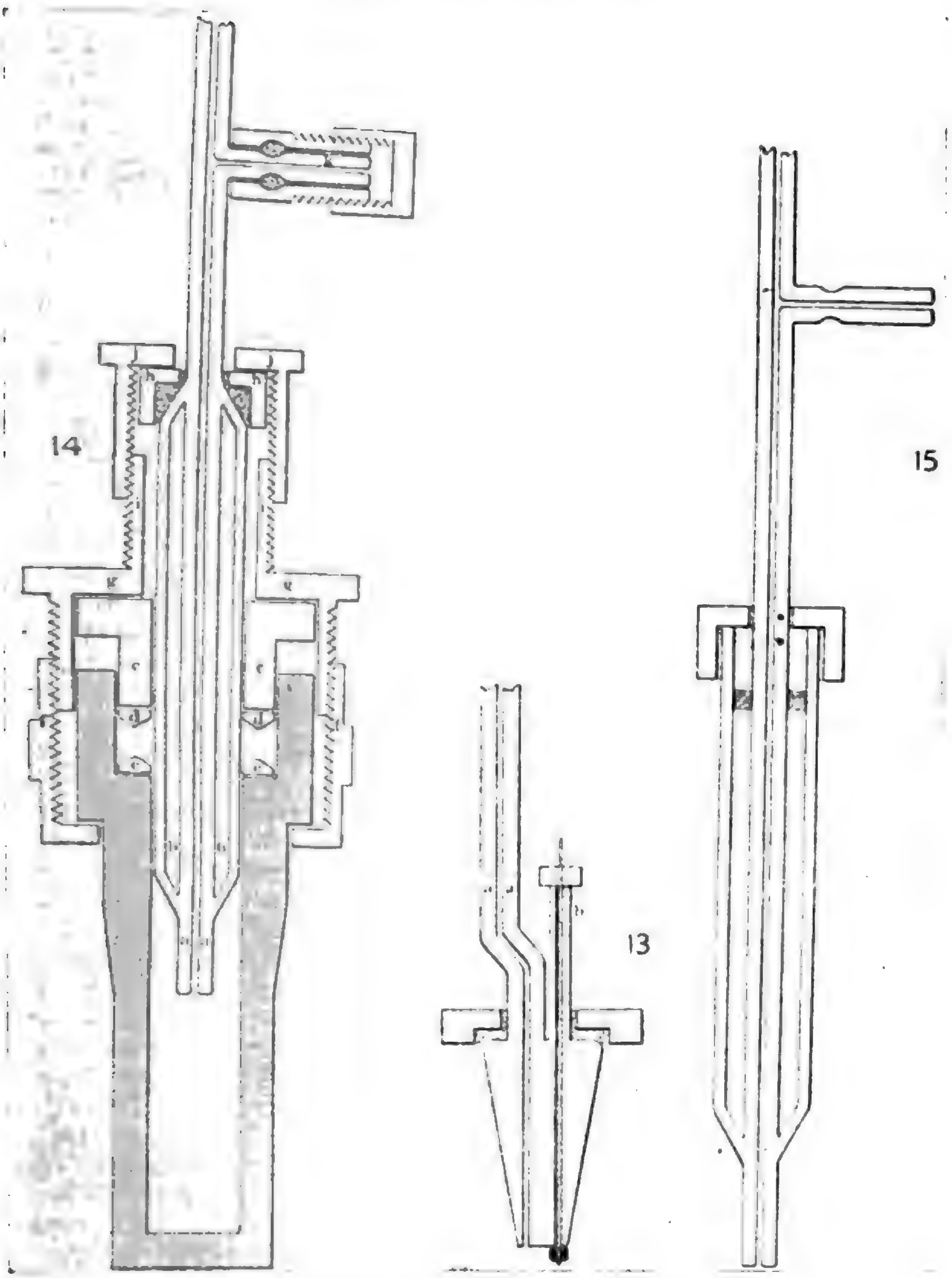

FIG. 48. ONE OF THE FORMS OF OSMOMETER USED BY MORSE.

13. Solid glass stopper for use with substances which attack metals.

a, Manometer tube.
b, Vent for solution, closed by valve at lower end of stopper

14. Glass manometer attachment for cells with straight necks.

a, Manometer with straight tube fused to lower end.
b, Space between manometer and glass tube.
c, Brass ring.
d, and *e*, Porcelain rings for compressing packing.
f, Brass collar.
g, *h*, *i*, and *j*, Brass pieces with which to close the cell, and also to adjust initial pressure.
k, Vent for solution.

15. Glass manometer attachment for cells with straight necks. Like that of Fig. 14, except that the glass tube is left open at the top and then closed with a brass cap and litharge-glycerine cement.

(Morse, 1914, p. 25; Carnegie Institution of Washington.)

osmotic pressure as that of the inner solution, so that no movement of the meniscus takes place. The concentration of the sugar solution can then be ascertained by an appropriate method, say by specific gravity or rotatory power, and its osmotic pressure is obtained from the measurements of Morse and others. The method is only applicable when the membrane is not easily permeable to the solute whose osmotic pressure is to be determined, and it must obviously not be acted on chemically by solvent or solute in contact with it. According to Walden (1892, p. 708) such membranes are permeable to nearly all inorganic salts. The substances tested by Fouard were lactose, glucose, mannite, asparagine, and quinine tartrate. Apparently the tannin-gelatine membrane was impermeable to these, but it is the most permeable of all the precipitation membranes tested by Walden (see page 113 above); the least permeable was that of copper ferrocyanide.

Vapour Pressure.—That a solution of any substance must have a lower vapour pressure than that of the pure solvent can readily be seen by the following consideration due to Arrhenius (1901, p. 33). Suppose two vessels, W and S (Fig. 49), situated in a closed space filled with air. W contains a dilute solution of a non-volatile solute in water, and S a stronger solution of the same solute. Water will pass from W to S, since the air may be regarded as a semi-permeable membrane, permeable to water as vapour, impermeable to the non-volatile solute. The pressure of water vapour over W must, therefore, be greater than over S, otherwise it would not pass from the one place to the other. Further, suppose that W and S, instead of being in separate vessels, are in one vessel but separated by a membrane, permeable to the solvent, impermeable to the solute. The water, as we know, passes to the stronger solution until the osmotic pressure of the two is the same. Now, if the pressure of water vapour were greater over S than over W, water would continually distil over to W and pass through the membrane to S, equilibrium would never be attained, and we should have a "perpetually automatic cyclic process, *i.e.*, a *perpetuum mobile*, which would perform work at the expense of the heat of the environment, which is contrary to the second law of thermodynamics" (Nernst, 1911, p. 132).

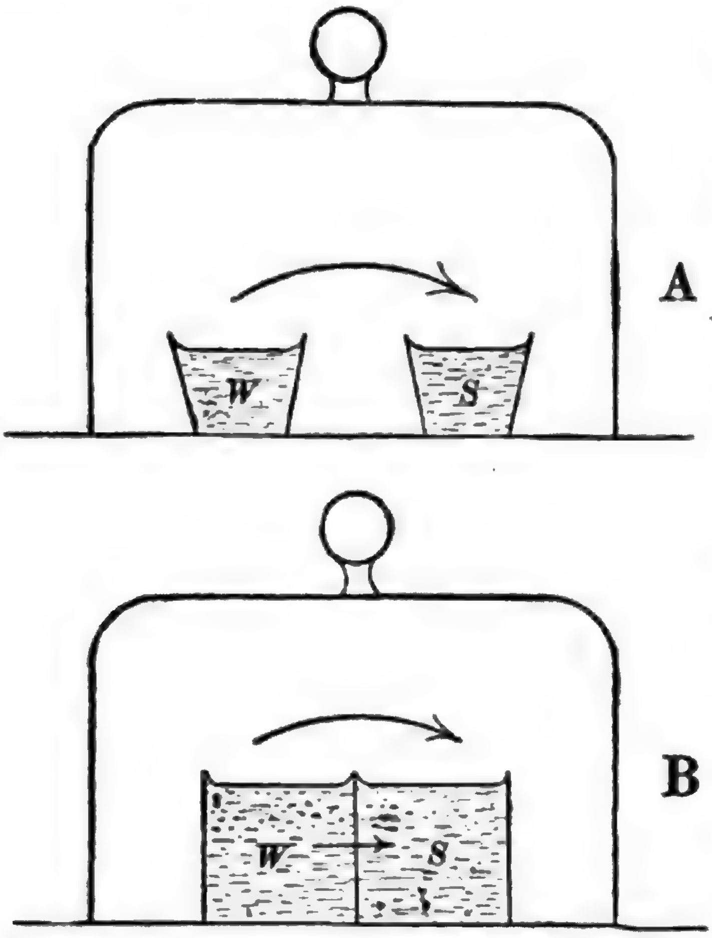

FIG. 49. DIAGRAMS TO ILLUSTRATE THE RELATION OF VAPOUR PRESSURE TO OSMOTIC PRESSURE.

W, Water.
S, A solution in water.

In *A*, the liquids are separated by air. In *B*, there is also a semi-permeable membrane, with which they are both in contact.

(After Arrhenius.)

The method of calculating the exact quantitative relation between vapour pressure and osmotic pressure is beyond the scope of this book, and may be found in that of Nernst (1911, pp. 132-137).

In practice, various methods of determining the vapour pressure of a solution are adopted. It may be measured directly by introduction of the solution into a Torricellian vacuum and measuring the fall of the mercury column, or by a differential method, determining the difference of pressure over the solvent and the solution. An apparatus for use in physiological work is described by Friedenthal (1903). The method has the disadvantage that the solutions are *in vacuo*, so that dissolved gases must be removed previously; but, on the other hand, it can be used at the temperature of the organism from which the solutions were obtained, an advantage over the freezing point method. Another method is that suggested by Ostwald and investigated by James Walker (1888). This depends on the fact that, when an indifferent gas is bubbled through a solution, the amount of the solvent removed by the gas is proportional to the vapour pressure of the solution. This method was employed by Berkeley and Hartley (1906, 2) to compare the vapour pressures of cane-sugar solutions

with the osmotic pressures obtained by the direct method. Several improvements were introduced in order to increase its accuracy. Another method is that of the dew-point, as used by M'Bain (1920).

When great sensibility is not required, Barger's method (1904) will be found very useful and easily carried out. Suppose that, in Fig. 49 (upper figure), we have a means of observing the changes in volume of the two solutions, and that we take as one of them a solution whose osmotic pressure is known, say cane-sugar, and that we change its concentration until no change occurs, on standing, in the volume of either of the solutions. Then the vapour pressure of the unknown solution is equal to that of the known sugar solution. Barger introduces alternate drops of the two solutions into a capillary tube, and observes the change in length of the various drops by measurement under a microscope.

It is clear that much time is saved by knowing beforehand the approximate osmotic pressure of the solution to be measured. In an application of this method to solutions of Congo-red (1911, ii. p. 233), I found no difficulty in distinguishing between concentrations of 0·020 and 0·023 molar.

The *boiling point* of a solution also depends on its osmotic pressure, and this method is frequently in use by chemists, but is rarely applicable to physiological problems on account of changes produced by the high temperature required.

On the other hand, the method of *freezing point* determinations is of great value, although not so sensitive as direct measurements. A decimolar solution in water lowers the freezing point by only 0°·184, so that a very sensitive thermometer must be used. In fact, 0°·001, a quantity difficult to measure with accuracy, corresponds to an osmotic pressure of 0·012 atmosphere, or about 9·1 mm. of mercury, a pressure easy of measurement, especially with a manometer containing a liquid of low density.

Solutions which have the same osmotic pressure have the same freezing point; for the freezing point is that temperature at which the solid solvent (ice) and the solution are capable of existing together, so that they must have the same vapour pressure, otherwise isothermal distillation would occur. Solutions have a lower vapour pressure than the pure solvent, hence the ice with which they are in equilibrium at their freezing points must have a lower vapour pressure than pure ice in equilibrium with water, in other words, it must be at a lower temperature.

It is scarcely necessary to remind the reader that ice has an appreciable vapour pressure, which decreases as the temperature falls, theoretically as far as absolute zero, at which temperature water vapour, like all gases, ceases to exist as such. This fact enables desiccation of tissues to be carried out below their freezing points, as in the method of Altmann (page 17 above).

In connection with the measurement of the freezing points of solutions there are two important laws to be kept in mind. The law of Blagden (1788) states that the lowering of the freezing point is proportional to the concentration of the solution, and that of Raoult (1883) states that equimolecular quantities of various substances in the same solvent lower its freezing point by the same amount.

For further theoretical treatment see Nernst's book (1911, p. 146), and for practical details of the methods used, see Findlay's monograph (1906, pp. 110-123), Nernst's book (1911, pp. 259-263), and the monographs of Raoult (1900-1901). Guye and Bogdan (1903) have modified the ordinary Beckmann apparatus in such a way as to make it available for smaller volumes of solutions, 1·5 c.c. instead of 10-20 c.c. This renders the apparatus of more use in physiological work, where it is not always possible to obtain sufficient liquid for the usual form of apparatus. A further modification, by which even less solution is required, is described by Burian and Drucker (1910). It appears, nevertheless, to give accurate results.

The value in degrees by which the freezing point of a solution is lower than that of water is denoted by the sign Δ.

There is still another method of measurement of osmotic pressure which has been used for physiological liquids, viz., that of the effect of dissolved substances on the *critical solution temperature.* Many liquids are able to dissolve each other to a limited extent, as, for example, phenol and water. Above a certain temperature these two liquids are miscible in all proportions, but, as the temperature falls, phenol separates out as a distinct phase in an opalescence to begin with. This temperature is altered by dissolved substances and in proportion to their

molecular concentration. For further details, the reader is referred to the paper by Timmermans (1907), and for the application to urine, the paper by Atkins and Wallace (1913), and for all methods of determining osmotic pressure, the valuable work of Hamburger (1904).

OSMOTIC WORK AND VOLUME ENERGY

To increase the osmotic pressure of a solution requires the performance of work just as the compression of a gas does. The amount of work depends, of course, on the volume of the solution compressed as well as on the pressure to which it is raised. It is, just as in the case of a gas, as described on page 33 above, equal to

$$RT \log_e \frac{p_2}{p_1}$$

for one gram-molecule, where p_1 and p_2 are the lower and higher pressures respectively; and n times this quantity for n gram-molecules.

The osmotic pressure of a solution can be raised by removal of part of the solvent in any manner, and it follows, from the second law of energetics, that the work done is identical in all cases (Nernst, 1911, p. 19), provided that the process is isothermal. Suppose that a part of the solvent is removed by evaporation, it can be shown by a simple process, details of which will be found in the book by Nernst (1911, pp. 132-135), that the work done is also expressed by the formula

$$P\frac{m}{s},$$

where m is the molecular weight of the solvent, s the specific gravity of the solution, and P the osmotic pressure of the solution.

The foundation of the general theory can best be grasped by the following imaginary model, based on the considerations of van't Hoff (1887). In a vessel, W (Fig. 50), containing a solution, S, is a cylinder, C, closed below by a membrane, impermeable to the solute, permeable to the solvent. The cylinder contains a more concentrated solution of the same substance, and is fitted with a movable piston on which weights can be placed so that the osmotic pressure due to the difference in concentration of the two solutions is balanced and the system is in equilibrium. A further weight is then placed on the piston; the result is that water is driven out through the membrane, so that the osmotic pressure is raised. In doing this, the weight falls through a certain height, thus doing a definite amount of work on the solution. If the added weight is removed again, water will enter, raising the original weight and so doing external work. We see thus that solutions, like gases, possess volume energy, which can be taken in or given out.

An important physiological application of this fact is that, when a secretion, such as urine, is formed at a higher osmotic pressure than the blood, work must be done, and that the work can be calculated.

OSMOTIC PRESSURE AND VELOCITY OF REACTIONS

In the inversion of cane-sugar by acid, when concentrated solutions are taken, the rate is found to be not in accordance with the law of mass action, "that the rate of change is proportional to the active mass of the substance taking part in the reaction." That is, if we understand by "active mass" the actual concentration in gram-molecules per litre. But Arrhenius has shown (1899) that, if we substitute for "active mass," in the above statement, the words "osmotic pressure," the experimental results agree with the law. As Mellor (1904, p. 283) puts it: "The osmotic pressure of cane-sugar in solution, kept at a constant temperature, is proportional to the number of collisions of the sugar molecule with the 'semi-permeable' wall of the containing vessel. Again, the amount of sugar inverted in unit time will be proportional to the number of collisions of the sugar molecule with the molecules, or rather the ions, of the acid. But

the amount of acid in the solution is constant, and consequently the number of collisions of the molecules of sugar with the molecules of the acid will be proportional to the osmotic pressure of the sugar molecules. In other words, the velocity of the reaction will be proportional to the osmotic pressure of the sugar molecules." As we have seen, in fact, the actual volume occupied by the sugar molecules must be taken into account, as was pointed out by Cohen (1897).

HYDRODIFFUSION

Substances in solution always wander from a place of higher to one of lower concentration. This is known as "*diffusion*" or "hydrodiffusion," and, according to the kinetic theory, is brought about by the constant movement of the molecules.

The phenomena were investigated by Graham (1850), who showed that the rate varied with the nature of the substance. Later investigations showed that the rate was inversely proportional to the size of the molecule, and directly proportional to the difference of concentration between the two places between which diffusion was proceeding.

In fact, the law is completely analogous to that sometimes known as Newton's *Law of Cooling* or, more generally, "Law of Velocities." Any process, which is on the way to an equilibrium, becomes slower and slower as the final state gets nearer. The driving force becomes less and less. The law applies to reversible chemical reactions as well as to the transfer of heat, the flow of water along a tube connecting two cylinders of water, and so on.

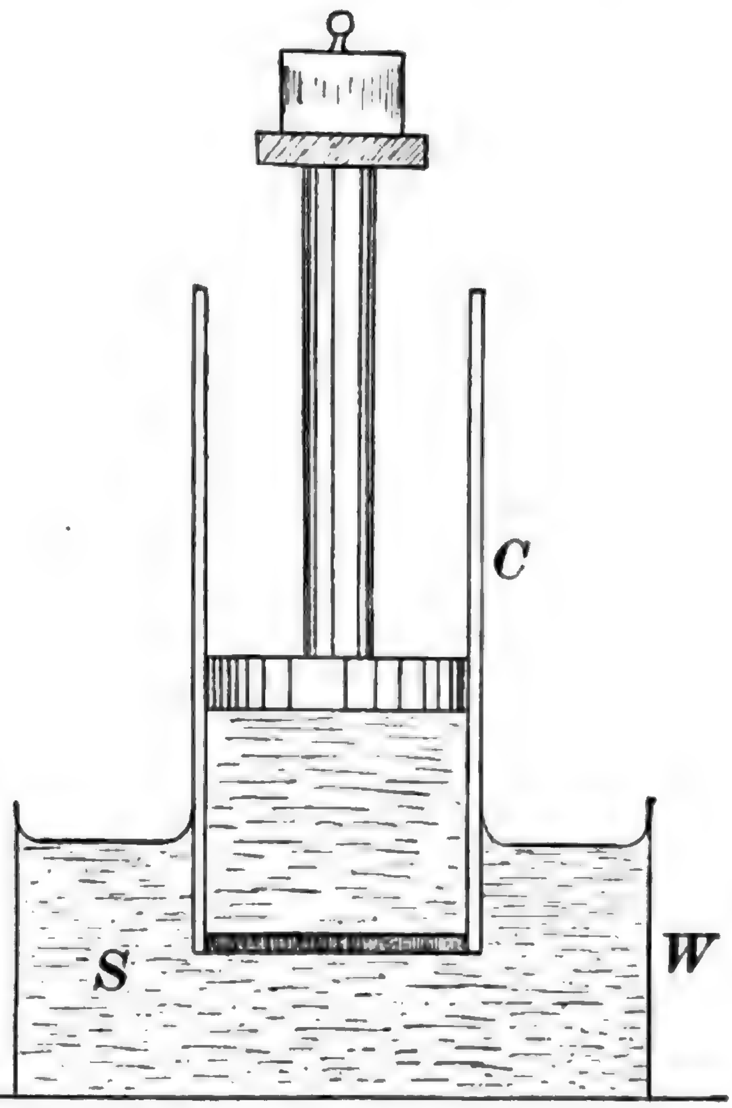

FIG. 50. SCHEMA TO ILLUSTRATE OSMOTIC WORK.

The cylinder (C) has an accurately fitting piston, and is closed below by a membrane semi-permeable as regards the solute in S. The solution inside the cylinder becomes more concentrated than that outside when the weight is placed on the top of the piston. To do this, the piston with the weight falls, thus doing work.

In the case of diffusion, the driving force is identical with osmotic pressure in solutions, and is completely analogous to the equalisation of differences of density in gases. In the latter, however, the process takes place very rapidly, while in a liquid it is very slow, owing to the enormous friction with which the moving molecules are met in the case of liquids.

It is interesting to calculate this friction from the osmotic pressure and the rate of diffusion, as can be done in a way analogous to Ohm's law. According to Nernst (1911, p. 152), it requires a force equal to the weight of $6{\cdot}7 \times 10^9$ kg. to drive one molecule (342 g.) of cane-sugar through water with a velocity of 1 cm. per second. We realise somewhat how slow a pure diffusion process must be. The following experiment described by Graham (1850, p. 462 of the Collected Edition) is instructive. A glass cylinder, 11 in. high, was filled to one-eighth of its capacity with a saturated solution of calcium bicarbonate, which also contained 200 gr. of sodium chloride in 8 cub. in. The jar was then filled completely with distilled water in such a way as not to disturb the lower layer, covered with a glass plate, and left to stand in a uniform temperature for six months. Samples of different strata were then removed by a syphon, and it was found that equality of concentration had not been attained, even in so long a time. The ratio of the concentrations of the sodium chloride in

the top and bottom layers was as 11 to 12, while that of calcium bicarbonate was as 1 to 4. In another set of experiments (p. 557 of Collected Papers), it was found that in fourteen days the concentration of sodium chloride at the top of a column of 127 mm. was only 1/22 of that at the bottom, while sugar was only just to be detected at the top in that space of time, the uppermost 50 c.c. of solution contained only 0·005 g. of glucose.

The different diffusion rates of various substances may give rise temporarily to considerable differences of osmotic pressure between two solutions in an osmometer, even when the two solutions are actually of equal osmotic pressure to begin with, and separated by a membrane permeable to both solutes. Sodium chloride diffuses more rapidly than magnesium sulphate, so that, if we take isotonic solutions on the two sides of a membrane permeable to both solutes, the former salt will diffuse through the membrane faster than the latter, the molar concentration and osmotic pressure of the sodium chloride solution will diminish, while that of the magnesium sulphate will increase, and water will pass to the latter. The difference in concentration is, of course, only temporary, but may give rise to considerable changes in osmotic pressure, and is of importance in the process of absorption from the alimentary canal.

When we have a solution of an electrolytically dissociated salt in contact with water, if the anion and cation move at different rates, it is clear that there will be a difference of potential between the front and back of the advancing surface of the diffusing column, the faster moving ions giving the sign of their charges to the front layer. Owing to electrostatic forces, the one set of ions cannot outdistance the other set further than their kinetic energy can carry them in opposition to the electrostatic attraction. (For the magnitude of these forces see the calculation by Arrhenius on page 179 below.) This phenomenon is a possible source of potential differences in tissues, and will be discussed later in Chapter XXII.

OSMOTIC PRESSURE OF COLLOIDS

The osmotic pressure of a solution is found to be, by whatever method it is measured, in direct relation to the molecular concentration. If a molecule is dissociated in any way, electrolytically or hydrolytically, each fraction acts as an element, equivalent osmotically to a molecule. Similarly, if there is association of molecules, the associated group behaves as a single molecule. The measurement of osmotic pressure is thus the most valuable means of determining the actual molecular concentration of a given solution.

Have we then any reason to limit the powers of giving an osmotic pressure to associations of a small number of molecules and deny it to those where a larger number are associated, as in colloids? Or at what particular number does osmotic pressure cease? Some substances, moreover, as we saw in Chapter IV., owe their colloidal properties to the fact that their single molecules or ions are too large to pass through parchment paper. If colloids have no osmotic pressure, it must be denied also to some molecules, so that we may again ask, at what molecular dimensions does it cease?

Any colloidal solution which remains in permanent suspension consists of particles in perpetual Brownian movement, precisely similar to the molecular movement postulated by the kinetic theory. Moreover, as shown by Perrin (page 85 above), each particle possesses the same mean kinetic energy as a molecule. If, then, this kinetic energy is the cause of osmotic pressure, it follows that colloidal particles must manifest it.

A brief consideration will show, however, that it cannot be expected to be great, at all events as far as the association of molecules constituting a suspensoid colloid are concerned. A true solution in decimolar concentration has an osmotic pressure of 1,702 mm. of mercury at 0°, as can be seen from the following calculation. One gram-molecule of a gas, at normal temperature and pressure, occupies a volume of 22·4 litres; therefore, to compress it to one litre, the volume of a solute in molar solution requires, by Boyle's law, a pressure of 22·4 atmospheres, or 17,024 mm. of mercury. But suppose that the same number of molecules as those in a decimolar solution are aggregated in masses of 500, then the solution, although containing the same amount of total solid, will have only 0·002 times the number

of active elements, or effective molar concentration, and its osmotic pressure will be only 3·4 mm. of mercury.

In the case of substances which are colloidal on account of the large size of their single molecules, as appears to be the case with hæmoglobin, it is impossible to obtain solutions of any great molar concentration. According to its content in iron, the molecular weight of hæmoglobin is 12,000 to 14,000, so that a 0·01 molar solution would contain 12 per cent. of solid. Colloidal solutions of such strength cannot often be obtained, and a decimolar solution would be solid.

The above considerations appear to me to place a difficulty in the way of accepting Roaf's view (1912, 1) of a cell membrane semi-permeable only as regards colloids. Plant cells usually contain solutions with an osmotic pressure of 4·5 atmospheres, which is that of a 0·2 molar solution; if a protein salt, even of so low a molecular weight as 2,000, is to afford this pressure, a 40 per cent. solution would be necessary. This is higher than the total solid content of protoplasm. More difficulties seem to be attached to this view than to that of a true semi-permeable membrane, although it is suggested as a simpler one. If we are to admit semi-permeability as regards glucose, or non-electrolyte crystalloids, it is not a great step to extend it to certain salts or even acids and alkalies.

The first clear proof that colloidal solutions have a measurable osmotic pressure was given by Starling (1896 and 1899) in the case of the colloids of blood serum. A portion of serum was filtered through gelatine by pressure; this filtrate contained all the crystalloid constituents of the serum, since gelatine holds back the colloids only. The filtrate was placed in an osmometer with a gelatine membrane, while on the other side of the membrane a portion of the unfiltered serum was situated. Any difference in osmotic pressure observed must be due to difference of molar concentration, and this again only to substances in the colloidal state. A value of 30-40 mm. of mercury was obtained. This is important in relation to the formation of urine.

Moore and Parker (1902) measured the osmotic pressures of egg-white, serum, and soaps, Moore and Roaf (1907) those of serum proteins, gelatine, and gum acacia. Hüfner and Gansser (1907), and Roaf (1910), independently, made exact determinations of that of hæmoglobin. Samec and Jencic (1915) show that "soluble starch" has an easily measured osmotic pressure. The valuable "protein studies" of Sörensen (1917) contain a detailed investigation of egg-albumin; M'Bain's paper (1920) of that of soaps.

Some confusion has arisen as to the genuine nature of the osmotic pressure obtained in the case of colloidal solutions on account of the difficulty of ensuring the absence of electrolytes or other impurities of low molecular weight. It was thought that, in some way, these foreign substances, although capable of free diffusion through the membrane, might be held back by the colloid and thus afford the osmotic pressure observed. Consideration will show that this cannot be the case. If these foreign substances are in chemical combination with the colloidal one, they are obviously part and parcel of the colloidal particles, and not to be reckoned as impurities. Even if merely adsorbed, they are fixed for the time on the surface of the colloidal particles, and are inseparable from the colloidal elements to whose molar concentration the solution owes its pressure—they are, in fact, not free to exercise their own osmotic pressure; while that due to the colloidal substance will rather, if anything, probably be slightly decreased, if the impurities are salts, owing to the increased aggregation of the colloidal particles. If, again, these foreign substances are free in solution, they will diffuse until equal in concentration on both sides of the membrane, and therefore inactive osmotically. There is, however, one special case to which reference has already been made (page 120 above), where both the colloid and the diffusible substance are electrolytes. But here the concentration of the diffusible salt becomes *less* in the presence of the colloid, so that it leads to a *fall* in the apparent osmotic pressure. Diffusible substances do not account for the osmotic pressure of colloids.

The osmotic pressure of hæmoglobin corresponds to its molecular weight, calculated on the basis of its iron content. Hüfner and Gansser (1907, p. 209).

Moore and Roaf (1907, p. 63) noticed that the addition of sodium hydroxide to a protein solution caused the osmotic pressure to rise, and interpreted the fact as due to the formation of a salt with smaller "solution aggregates" than the original protein. Now, in order to investigate the interesting and important phenomena shown by electrolytically dissociated salts, of which one or the

other ion does not pass through the membrane, it is better to take salts of which the molecular weight and chemical constitution are known, since quantitative results are easily obtained. Many of the aniline dyes with large molecular weight answer this requirement. In the work done by myself (1909, 1911), Congo-red and related dyes were found the most useful. It is necessary to devote some consideration to this question, since the conditions are rather complex, but salts of this nature are of frequent occurrence in the cell, and play an important part therein.

The exact chemical constitution of Congo-red is not material for the present purpose, except that the coloured ion is the anion and, being a substituted di-sulphonic acid, combines with two sodium ions. The anion is, of course, the one to which the parchment paper membrane is impermeable. Measurements of the electrical conductivity of these dyes show them to be electrolytically dissociated to a considerable degree, so that the question to be answered is whether the sodium ions are active osmotically when the membrane used is permeable to them. It might indeed be supposed that these ions would pass through the membrane to such a distance that their osmotic pressure was balanced by the electrostatic attraction of the non-diffusible ions within the membrane, and that this fact would render ineffective any pressure due to the kinetic energy of these ions on the opposite side of the membrane. It must be confessed that the conditions are difficult to grasp in thought, but it will be remembered that the osmotic pressure produced by the non-diffusible substance, consisting of the anions and the non-dissociated part of the salt, shows itself in virtue of the mechanical constraint exerted by the membrane, which allows water to pass through freely, while holding back the substances named. In a similar way, the sodium ions are held back by the attraction of the opposite ions, which themselves are held back by the membrane, so that the membrane itself must actually bear the pressure of both kinds of ions. Or, to put it in another way, the pull of the anions on the cations could not be effective unless the constraint of the membrane gave the former a support to pull against.

Experimental evidence, in any case, shows that all the ions actually present are osmotically active. Vapour pressure measurements made by the method of Barger, described above (page 155), gave the same values as direct measurements with a parchment paper osmometer (Bayliss, 1911, p. 233). Now this vapour pressure method gives the total molar concentration of the solution, including that of the sodium ions, and therefore the parchment paper membrane does so also. A still simpler proof that the diffusible ions are really active, is to take the dye, "Chicago blue," in which the anion, like that of Congo-red, is a complex sulphonic acid, but in this case there are four sulphonic acid groups in the molecule, so that it combines with four sodium ions. If the latter were inactive, the osmotic pressure with a parchment paper membrane would be the same as that of an equally concentrated solution of Congo-red, since the concentration of the non-diffusible anion is identical; in point of fact it is found to be double, hence the sodium ions play their part.

The matter is, however, not quite so simple. Although all the ions that are present must be osmotically active, the numerical values of the osmotic pressure, whether measured directly or by vapour pressure, are much less than would be the case if the dissociation were of the usual simple kind of such an inorganic salt as sodium chloride. The reason for this has not yet been satisfactorily made out, but there seems to be no doubt that it depends on the formation of complex, aggregated ions. The remarkable fact is that electrical conductivity measurements give no evidence of a less total number of charges than if no aggregation existed. The complex ions appear to possess the same number of charges as if their constituents were free.

The way in which the electrostatic forces at the membrane influence the distribution of diffusible salts between the two sides of the membrane has been referred to above (page 120). It is found that, suppose the dye is a sodium salt, and the diffusible salt is sodium chloride, the distribution is such that there is always less of the sodium chloride within than without. The explanation is, no doubt,

that in equilibrium, there must always be equal concentration of non-dissociated sodium chloride on both sides, since it is freely diffusible and there are no electrostatic forces to prevent its equal distribution. To ensure this, the total amount of sodium chloride present must be less inside, on account of the fact that its dissociation is lowered by the presence of an ion ($Na^{\cdot}$), which is common to the two salts within the membrane.

At first sight it seems strange that salts which have no ion in common with the dye are also affected in the same way. The reason is that, when equilibrium is established, there are present, inside and out, both kinds of the diffusible cation in the same ratio. The layer of $Na^{\cdot}$ ions, arising from the dissociation of the dye salt, and situated on the outside of the double layer at the membrane, must not be thought to be composed of the same individual ions—there is perpetual interchange with those in the body of the solution. Suppose now we place a solution of potassium chloride outside; the sodium ions, since they are kept in place merely by virtue of their positive charges, will naturally interchange with potassium ions of the outer solution, so that, to begin with, the outer layer at the membrane will consist of both $K^{\cdot}$ and $Na^{\cdot}$ ions; these, in their turn, interchange with the $Na^{\cdot}$ ions in the solution within the membrane, so that finally there will be the same relative distribution of total diffusible salt as if sodium chloride had been taken. Naturally there will also be present a certain proportion of the potassium salt of the dye in place of a part of the sodium salt originally present. An important point to be noticed is that the *ratio* of sodium to potassium will be the same inside and outside, as indeed I have found experimentally to be the case. It follows, as already pointed out, that a membrane impermeable merely to colloids will not account for the unequal ratio of sodium and potassium inside and outside the red blood corpuscles. The membrane must be impermeable to these also.

This formation of a double layer at the membrane, as pointed out by Laqueur and Sackur (1903, p. 203), should give rise to a considerable difference of potential between the two sides of the membrane. Theoretical considerations show that it will be expressed by the same formula as that deduced by Nernst for the potential of metallic electrodes, viz. :—

$$\frac{RT}{nq} \log \frac{c_2}{c_1},$$

where R and T have their usual significance, q is the charge on one gram-equivalent of the diffusible ion concerned, n is the number of these gram-equivalents, c_2 is the concentration of this ion inside the membrane, and c_1 its concentration in the outside solution. Direct measurements made by myself (1911, pp. 243-248) confirm the correctness of the formula as applied to the case in question. The reader will recognise this formula as being the same as that for the isothermal compression of a gas or the concentration of a solution, the only difference being that, as we are dealing with electric charges, we have to introduce q, in order to give the correct numerical values to our result. In other words, the number in gram-equivalents of the ordinary formula has to be changed into the number of charges on these gram-equivalents. R, the gas constant, must also be expressed in electrical units. The way in which the formula is obtained is described below (Chapter XXII.).

It is not to be forgotten that the results given in the present section apply not only to dyes, but to all salts of which one ion is held back by a membrane, permeable to the opposite ion. They apply to salts of proteins and also to non-colloidal electrolytes, if the membrane is impermeable to one only of their ions. This latter case has been discussed by Ostwald (1890). The considerations with regard to interchange of ions form also the explanation of the experiments of W. A. Osborne (1906) on the interchange of ions between colloids and salts.

Since dyes of the molecular weight of Congo-red give considerable osmotic pressures, owing to the fact that their molecules are only just sufficiently large to be unable to pass through parchment paper, they form very useful substances for the investigation of many problems relating to osmotic pressure. The difficulty of preparing reliable copper ferrocyanide membranes is avoided. A 0·01 molar solution of Congo-red has an osmotic pressure of 170 mm. of mercury, and that of Chicago-blue is nearly double. There is one practical point to be taken care about, if permanent readings are to be expected, when dyes with an indiffusible anion are made use of. The free acid is insoluble, and although it forms a colloidal solution when free from electrolytes, it is precipitated by traces of them. If the outer water is exposed to the air, it will absorb carbon dioxide; this, being diffusible, obtains access to the interior of the osmometer, and, although a weaker acid than that of the dye, it will slowly decompose the salt, by mass action, owing to the precipitation of the free acid out of solution, while the sodium carbonate diffuses away to the outer water. The fact

itself was noticed by Graham (1861, p. 217) in connection with the sodium salt of "albumen," where it was found that all the sodium diffused away in process of time and was found in the outer water in combination with carbon dioxide derived from the air. The same thing happens with the salts of caseinogen. It is necessary to give this warning, since various incorrect statements have been made on the basis of experiments in which this factor was ignored. It is, for example, no proof of hydrolytic dissociation when sodium is found to have diffused out.

The osmometer of Moore and Roaf (1907), with the additions described by myself (1909, i. p. 271), will be found suitable for the investigation of colloidal solutions. The platinum lining is rarely necessary; it will be found sufficient to have the inside electro-gilt. The membrane may be of parchment paper, or of this impregnated with gelatine, collodion, etc.

Many proteins, as we have seen (page 104 above), take up water by *imbibition*. In theory it would seem, therefore, when a certain molar solution is made, that the solution is really more concentrated than was intended, owing to the taking up of water by the colloid, which water is then no longer free as solvent. The osmotic pressure would, for this reason, be higher than the theoretical one. It is difficult to state how far this is actually the case, since we are so much in the dark as to the true molecular weight of proteins. The case of hæmoglobin, which has an osmotic pressure in agreement with its molecular weight, suggests that the effect of imbibition is negligible. Some measurements of the osmotic pressure of the sodium salt of caseinogen made by myself (1911, i. p. 234) agree with the molecular weight assigned by Laqueur and Sackur (1903, p. 199). It may be that, although each molecule of the protein takes up a considerable number of water molecules, the total number of protein molecules present is too small to affect appreciably the molar fraction of the water, which is always present in excess. Pauli (1910, p. 485), however, is of the opinion that the process of imbibition plays an important part in the apparent osmotic pressure of proteins.

Since the manifestation of osmotic pressure is an aspect of the kinetic energy of particles in motion, which also shows itself in the power of diffusion through a liquid, it is interesting to note that Svedberg (referred to by Arrhenius, 1912, p. 27) found that a certain gold hydrosol had a diffusion constant of 0·27 per day; in the same units, chlorine, bromine, and iodine have respectively values of 1·22, 0·8, and 0·5. There is, then, more difference between the rates of chlorine and iodine than between those of iodine and of gold particles.

RELATION TO CELL PROCESSES

Living cells, as we saw in the previous chapter, are surrounded by a semi-permeable membrane, so that it is obvious that the osmotic pressure of the solution outside, compared with their own osmotic pressure, is of great importance in many ways.

The osmotic pressure in the interior of such an organism as an Amœba must be higher than that of the fresh water in which it lives. Hence, if the cell is covered by a semi-permeable membrane, water is continually being taken up into its substance. According to Stempell (*Zool. Jahrb. Abt. Zool.*, xxxiv. (1914) pp. 437-478), it is the function of the contractile vacuoles of these organisms to get rid of, periodically, the water which has entered in this way.

There is, we may note in the next place, an important difference between vegetable and animal cells. The former, surrounded by a tough cellulose envelope, are usually surrounded by water or by a considerably hypotonic solution; in this way their internal osmotic pressure is uncompensated and maintains a state of tension or "*turgor*" in the cell, serving to keep up the more or less rigid condition of living plant structures necessary for their satisfactory exposure to air and light.

Animal cells, on the contrary, are, as a rule, free to change their dimensions by taking or giving up water. In order that they may remain in a normal state, therefore, they must be surrounded by an isotonic solution. Now, any substance in appropriate concentration will make an isotonic solution, provided that the cell membrane is impermeable to it. On the other hand, there are very few substances which have no action on the cell beyond that due to their osmotic pressure. Perhaps cane-sugar has the least action, but, as we saw above (page 125), it is not a completely indifferent substance. The effects of solutions which are merely due to their osmotic pressure are accordingly rather difficult to investigate. In certain cases, however, the state of affairs is quite clear.

We may take first an interesting fact discovered by Dale (1913). The uterus of the guinea-pig is a very useful preparation for researches on the action of *drugs on smooth muscle* tissue. When suspended in isotonic saline solution (Ringer's fluid), it responds by contraction to the addition of various drugs, *e.g.*, β-iminazolylethylamine. Suppose that the concentration of the solution in sodium chloride is raised from the normal 0·9 per cent. to 1·1 per cent., the response is greatly decreased and is practically abolished at 1·3 per cent. If the osmotic pressure is raised by isotonic quantities of sodium sulphate or cane-sugar, the effect is identical, so that it appears to be one of tonicity alone. The reverse action may be produced by dilution, even from 0·9 per cent. to 0·85 per cent., so that the response to stimulant drugs is markedly increased. Dilution with isotonic cane-sugar has no effect, but urea solution acts as pure water, since the cells are permeable to it. When the action of a drug is to produce relaxation of a tonic state, as in the case of adrenaline on the virgin uterus of the cat, the effect of *increase* of osmotic pressure is to *increase* the inhibitory action and of decrease of osmotic pressure to diminish it. The tonus itself is also inhibited by rise of osmotic pressure and increased by addition of water.

We have already discussed briefly the two typical cases of the cells of the kidney and of striated muscle, as investigated respectively by Siebeck (1912) and by Beutner (1912, 1913). The *volume of the cells* was found to be in exact relationship to the osmotic pressure of the solution outside them.

Diminution in the volume of cells by loss of water must have the effect of *increasing the internal concentration* of substances to which the membrane is impermeable. By mass action, reactions of which these substances are components will be accelerated, and increase in volume by absorption of water will retard such reactions.

An interesting case is that of yeast. The cells of this organism, owing to the store of glycogen which they contain, undergo a process of auto-fermentation, the enzymes present acting on the glycogen, first to form sugar and then to convert it to alcohol and carbon dioxide. It was found by Harden and Paine (1911) that, if the cells are placed in solutions which cause plasmolysis, the rate of auto-fermentation is greatly increased, no doubt by increase of concentration both of enzymes and of substrate. Solutions of various substances, if their osmotic pressure was the same, caused equal increase. If no plasmolysis resulted, either because the solution was isotonic with the cell contents, or because the cell membrane was permeable to the solute, as urea, no effect was obtained.

Perhaps one of the most obvious phenomena in which osmotic pressure plays a part is that of *secretion*. Let us imagine a vertical tube, closed at the lower end by a semi-permeable membrane and open at the upper end. Let it be filled with a solution of cane-sugar and placed with its lower end in water. Water will enter the tube by osmosis and cause a continuous flow of liquid over the top as long as any osmotically-active substance is present inside it. It will clearly be without effect on the result if the top of the tube is closed by a permeable membrane, or even by a membrane through which cane-sugar can pass, however slowly, so long as it passes more quickly than through the semi-permeable membrane at the other end. If such a tube, with a permeable membrane at one end and a semi-permeable membrane at the other end, be totally immersed in water, or a solution of less osmotic pressure than that contained inside it, a current will flow through it, carrying out the solute, until the osmotic pressure is equal inside and outside.

A mechanism of this kind exists in certain organs of plants, in which drops of watery secretion are formed at the apex of a column of cells. These organs are known as "hydathodes" and the cells have been shown by plasmolytic methods to increase in osmotic pressure as the apex is approached (Lepeschkin, 1906). The aerial hyphæ of the fungus *Pilobolus*, which secrete drops at their tips, have been also investigated by Lepeschkin (1906) and a similar mechanism found.

The phenomenon of bleeding at cut ends of stems, or *root pressure*, receives its explanation in a similar manner. The liquids in the root have a higher osmotic pressure than the very dilute solution in the soil, and, since the cells are provided with semi-permeable membranes, a flow of liquid takes place as in our glass tube model.

An important series of papers has been published by Demoor and his coadjutors (1907) on the relation of *secretory organs*, such as the liver, kidney, and submaxillary gland, to the osmotic pressure of the liquid perfusing their *blood vessels*. The discussion of some of these facts will be found in Chapter XI.; in this place one or two suggestive points only will be referred to.

The cells lining blood vessels, like other living cells, are no doubt subject to changes of volume in response to changes in the osmotic pressure of the blood. According to Demoor, the effect of this change in volume will be to alter the lumen of the vessel, so that, other things being equal, a fall in the osmotic pressure of the blood causes a swelling of the lining cells of the blood vessels and a consequent narrowing of the lumen. This fact has special application to the function of the kidney. In the case of the liver (1907, p. 32) it was found that the rate at which 1·5 per cent. sodium chloride passed through was greater than that of 0·6 per cent., while 0·9 per cent. was intermediate. A solution of a concentration of 0·6 per cent. became more concentrated and one of 1·5 per cent. became diluted in its course. So that it is clear that the cells take up water from a hypotonic solution and that the swelling so caused obstructs the circulation, and vice versa. How far the effect is due to the liver cells themselves and how far to the lining cells of the blood vessels is not quite clear, but it seems probable that the former is the chief factor. We call to mind that the liver capillaries send branches into the substance of the liver cells (Schäfer, 1902), so that the capillaries are devoid of walls in certain places. The reactions described disappear when the semi-permeability of the cells is destroyed by sodium fluoride.

Similar phenomena were found in the pulmonary circulation (page 50), and it seems probable here that changes in the volume of the lining cells of the blood vessels might play the chief part.

In the kidney, we meet with the same facts as regards the rate of flow of blood. The rate of secretion falls also with hypotonic solutions and rises with hypertonic, as had been observed by Starling (1899). But investigations on the changes of volume of the kidney show that the organ, as a whole, *swells* when a hypertonic solution is perfused, and vice versa (page 69). Owing to the complexity of the factors involved here, discussion of the question will best be postponed to Chapter XI.

We have seen why it is necessary for animal cells to be in contact with a liquid of the same osmotic pressure as themselves. There is evidence, moreover, that when exposed to the action of a liquid of a different osmotic pressure, they are able to *accommodate* themselves to a certain extent by change in their own osmotic concentration. For example, it appears that the cambium cells of trees, as the external pressure upon them increases, produce osmotically-active substances in order to raise their own osmotic pressure.

The *body fluids*, including the blood, of marine invertebrates, have the same osmotic pressure as the sea water in which they live, as shown by Bottazzi (1897). *Maia verrucosa*, a crustacean, for example, is placed in concentrated or diluted sea water, and it is found that the body fluid takes the same osmotic pressure as the solution; the following data from Fredericq's paper (1901) will show this:—

	Δ of	
	Sea Water.	Body Fluid.
	Degrees.	Degrees.
Normal	2·3	...
Concentrated	2·96	2·94
Diluted	1·38	1·4

The regulation is apparently effected through the cells of the gills. Since the changes in question do not permanently affect the animal, it is plain that

the cells must have altered their own osmotic pressure to compensate for the change in that of the body fluid.

The same behaviour is shown by the lower fish, the Selachians. But, as we ascend the scale of evolution, we find that the blood is maintained at a nearly constant osmotic pressure by regulative mechanisms. The following values from Bottazzi's article (1908) apply to marine organisms:—

Selachian fish - - -	$\Delta = 2^{\circ}\cdot26$
Teleostean fish - - -	,, $1^{\circ}\cdot04$-$0^{\circ}\cdot76$
Reptile, turtle - - -	,, $0^{\circ}\cdot61$
Mammal, whale - - -	,, $0^{\circ}\cdot65$-$0^{\circ}\cdot7$

In the Teleostean fish we find the regulative mechanism in process of development. Dakin (1908) found that the depression of the freezing point of the sea water at Kiel was $1^{\circ}\cdot09$, while at Heligoland it had risen to $1^{\circ}\cdot9$; correspondingly, that of the blood of the ray (a Selachian) rose in agreement. That of the plaice (a Teleostean), on the contrary, had risen from $0^{\circ}\cdot66$ to $0^{\circ}\cdot8$ only, *i.e.*, by 20 per cent., while that of the water had risen by 74 per cent. The cod is still more independent of the medium; when the Δ of the sea water rose from $1^{\circ}\cdot2$ to $1^{\circ}\cdot9$, that of the cod only rose from $0^{\circ}\cdot73$ to $0^{\circ}\cdot757$, by 3·9 per cent. only.

We notice that, as the power of osmotic regulation becomes more manifest in the animal scale, the Δ of the blood tends to be fixed at about $0^{\circ}\cdot6$, which is the value of that of the higher land vertebrates.

The advantage of a fixed osmotic pressure will be clear if we remember that it is due, almost entirely, to salts. Colloidal systems, such as protoplasm is, are especially sensitive to electrolytes, as we saw in Chapter IV., and fine adjustments of such processes are the more perfect, the greater the constancy of the electrolyte concentration of the medium in which they take place.

The regulation of the osmotic pressure of the blood to a constant value is shown in an interesting way by some observations of Cohnheim (1912, 1). Sweat contains a considerable amount of salts, having a Δ of about $0^{\circ}\cdot5$, according to Tarugi and Tomasinelli (1908). Now Cohnheim found that he lost a certain considerable weight in this manner by performing a mountain ascent in hot weather. This weight could only permanently be replaced if he drank water containing sufficient salts to replace those lost. Distilled water was rapidly lost again through skin and kidneys.

EFFECTS OF CELL METABOLISM

The chemical changes associated with those cell activities which result in the setting free of energy usually consist in the splitting up of large complex molecules into a greater number of smaller ones, such, for example, as the oxidation of one molecule of glucose into six molecules of carbon dioxide and six molecules of water. The osmotic pressure of a solution being in proportion to its molar concentration, it is clear that, neglecting the water, the osmotic pressure of a glucose solution would be raised to six times its value. The bearing of this fact on the formation of lymph in active organs will be seen presently.

The mere addition of *carbon dioxide to blood* raises the osmotic pressure of the latter considerably more than the molar concentration of the added substance would account for. Kovács (1902) states that addition of carbon dioxide to rabbit's blood raises the Δ in ten minutes from $0^{\circ}\cdot6$ to $0^{\circ}\cdot72$. The effect is due to a complex reaction with the salts of blood, which will be discussed in the next chapter.

LYMPH PRODUCTION AND ABSORPTION FROM TISSUE SPACES

It is rarely that the blood vessels lie in immediate contact with the tissue cells, whose food requirements the blood supplies and the products of whose metabolic changes it carries away. There intervenes a space, of varying dimensions, containing a fluid, the lymph, whose composition is very similar to that of the blood, minus its red corpuscles, although usually containing less protein. This lymph is being produced continually at a more or less rapid rate by transudation from the blood vessels, and carried back to the blood by means of the lymphatic vessels. Filtration is one of the factors concerned in its production, since the intravascular pressure is greater than that in the tissue spaces; but Starling

(1896 and 1920) has insisted on the importance of osmotic processes in addition. It is, in fact, clear that a rise in the osmotic pressure of the lymph, however this rise is produced, will result in the passage of water from the blood to the lymph, and an increase in the volume present. We see then why the amount of lymph flow from an organ is increased by activity of that organ. The energy required for activity is afforded by chemical processes which result in the production of a larger number of small molecules from larger ones, with a consequent increase in the molar concentration and osmotic pressure of the contents of the cells. These metabolic products diffuse from the cells into the lymph surrounding them, thus raising its osmotic pressure above that of the blood, with the result that water passes from the latter to the lymph and causes an increase in its volume.

Lymph contains only about half the protein content of blood plasma, and the blood vessels may be considered to be, normally, impermeable to the colloids, like the renal glomeruli. Thus there is an effective osmotic pressure of about half that of the colloids of blood, in fact, about 15 to 20 mm. of mercury. Where the blood pressure is higher than this value, there is filtration to the tissue spaces. Nearer the venous end of the capillaries the blood pressure is lower than the effective osmotic pressure, so that fluid is attracted in. In normal conditions, the two processes, with the assistance of the flow along lymphatic vessels, are balanced. If the osmotic pressure of the blood colloids falls, as in hydræmia, the effective filtration pressure is increased, and the length of the path along which filtration occurs is longer. Added to this, the osmotic pressure leading to reabsorption is lower, so that less fluid is taken up again. The two causes combined result in œdema.

When the arterial pressure falls from loss of blood, filtration is less, whereas reabsorption is unchanged, since the osmotic pressure of the blood colloids is at first undiminished. Reabsorption thus surpasses filtration, and fluid is added to the blood, tending to restore its volume (see also Starling, 1896, p. 321).

It will be clear that the above considerations play an important part in determining the nature of an appropriate fluid to replace blood lost by hæmorrhage, or to dilute it when concentrated by loss of water or plasma (Bayliss, 1916).

The observations of Yanagawa (1916) on the action of drugs on lymph flow should be consulted.

Use of the Expression "Osmotic Pressure"

It is, perhaps, well to make a few remarks with respect to the view held by some that osmotic pressure only exists in the presence of a semi-permeable membrane. If this is so, we are incorrect in speaking of the osmotic pressure of a solution under any circumstances except those in which it is separated from pure solvent by means of a membrane impermeable to solutes. When, therefore, that property of a solution which would cause it to show osmotic pressure under these special circumstances is determined by some other method, such as freezing point, another name must be used.

It is clear that such a practice, although perhaps in agreement with the original meaning of osmosis as used by Dutrochet, would give rise to much inconvenience, and even confusion. We need a word to express the total concentration of a solution in such elements as act as molecules in the sense of Avogadro's law, since the molar concentration does not afford the information in the case of electrolytes and colloids. It seems to me that we are quite justified, even in theory, in speaking of the osmotic pressure of the blood, for example, without any reference, even in thought, to a semi-permeable membrane. We mean to express those properties conferred by the kinetic energy of the molecules, or elements equivalent to them, of the solutes. In the presence of a semi-permeable membrane it would be shown as a definite pressure, capable of measurement by a manometer; but the phenomenon which causes this pressure is always there and leads to diffusion, amongst other things.

This denying of the existence of osmotic pressure except in relation to a membrane leads to the denial of its existence altogether, since we know of no perfect semi-permeable membrane.

No objection is made to the statement that the air in a vessel open to the atmosphere has a pressure of 760 mm. of mercury, although it is not to be detected unless the vessel is closed and provided with a manometer while the outer atmosphere is removed.

In the present book I shall continue to use the words "osmotic pressure," meaning thereby that property of solutions conferred upon them by the kinetic energy of the solutes.

The name "*tonicity*" is sometimes used, especially in reference to blood corpuscles and living cells in general, but it is not necessarily the same as osmotic pressure, unless we admit that the latter may vary according to the membrane used. For example, we say that a solution of sodium chloride is "isotonic" with mammalian blood corpuscles, because it produces no change in their volume. But we might add an equivalent amount of urea to this solution without making it less "isotonic" with the blood corpuscles, because their membrane is permeable to urea. On the other hand, its osmotic pressure is really doubled, as shown by

vapour pressure measurements. The word "isotonic" can only be used when the nature of the particular membrane is specified, and refers only to those constituents of the solution to which the membrane is impermeable; osmotic pressure refers to the total concentration, assuming that the membrane is impermeable to all the solutes, permeable to the solvent.

Macallum (1911, p. 617) appears to suggest that the van't Hoff-Arrhenius theory of osmotic pressure does not hold in physiological phenomena. The osmotic pressure of the cell-contents is said not to be given by the total concentration of electrolytes in the cell, because these may be concentrated by surface tension at the cell-membrane. This does not seem to me to be quite the correct way of putting the matter. Osmotic pressure is only shown by *free* electrolytes. In estimating, therefore, the osmotic pressure due to the potassium salts in a cell, that part of the salts adsorbed on surfaces must be left out of account. Although the actual concentration of potassium may be greater at the cell boundary, it does not follow that its osmotic pressure is any greater here, because it is concentrated on account of its property of lowering surface energy, and, to do this, it must be held in constraint by the surface, adsorbed in fact, and thus unable to possess the kinetic energy necessary for the manifestation of osmotic activity.

SUMMARY

When any substance is dissolved in a solvent, the solution, as compared with the pure solvent, behaves as if the solute were exercising pressure.

This pressure is known as "osmotic pressure," because, when the solution is separated from pure solvent by a membrane which is impermeable to the solute, but permeable to the solvent, it is found that the solvent passes to the solution, increasing its volume, by the process known for many years as "osmosis."

The existence of the pressure can be shown by connecting the vessel, containing the solution as above, to a manometer, so that increase of volume is prevented, and the manometer indicates the rise of pressure. Indirectly, the effect of the solute on the vapour pressure of the solvent, and the various phenomena dependent upon this, show the pressure exerted by the solute.

The amount of this pressure was shown by van't Hoff, on the basis of the experiments of Pfeffer and De Vries, to be identical with that which would be exercised by the solute if converted into gas and compressed to the same volume which it occupied in the solution.

Since the simple gas law only applies, even to gases, under limited conditions, it is not to be expected that it would apply to solutions, especially to concentrated ones, without correcting factors.

Such factors are present in van der Waals' "equation of state" as applied to gases and to pure liquids. They result from the considerations of the actual space occupied by the molecules themselves, so that the space left free for movement is diminished, and of the mutual attraction exercised by the molecules upon each other, by which the pressure due to their kinetic energy is reduced.

If similar additional correcting factors are introduced into the van der Waals equation to take account of the interaction between the molecules of the solvent and of the solute, an equation can be formed which expresses the osmotic pressure of solutions in general.

The kinetic theory of the origin of osmotic pressure satisfies physiological requirements better than other theories do.

Hydration of solute, or imbibition of solvent by it, has a negligible effect, except in the case of very concentrated solutions, owing to the enormous preponderance in number of the free molecules of the solvent in comparison with those fixed by the solute.

In practice, osmotic pressure is measured either directly or by methods depending on changes in vapour pressure, of which the depression of the freezing point of the solvent is that most frequently used. In the case of water, this value is called $\triangle$.

In whatever way the osmotic pressure of a solution is raised by removal of solvent, the same amount of work must be done to produce the same amount of change. The mathematical expression is identical with that for the isothermal compression of a gas.

It follows that, by appropriate means, a solution can be made to do work by dilution; the capacity factor of this work is the volume of the solution undergoing dilution. Solutions then, like gases, possess volume energy.

According to the kinetic theory, substances in solution must diffuse from places of higher concentration to those of lower concentration, until the same concentration is attained in both. Unlike gases, however, the process is extremely slow, owing to the great resistance met with.

Since the kinetic energy of a molecule, an ion, or a colloidal particle is the same, these elements are mutually equivalent as regards the production of osmotic pressure, which depends only on the molar concentration of the elements active. Colloidal solutions, therefore, must possess a true osmotic pressure, which is usually small, on account of the low molar concentration of such solutions in active elements.

Diffusible impurities play no part in the osmotic pressure of colloids, except in so far as they may affect the degree of dispersion of the particles of the colloidal state.

The osmotic pressure of electrolytically dissociated salts, of which one ion only is colloidal, requires special consideration. It is shown that the diffusible ions, although the membrane is permeable to them, play their full part in the production of osmotic pressure.

Certain special phenomena, of which explanation is given in the text, are present in such cases. A salt of which both ions are diffusible through the membrane, if added to the system, is found, when equilibrium is attained, to have distributed itself in such a way as to have a lower concentration in the presence of the colloidal salt. This happens whether the two salts have an ion in common or not. There is also a considerable potential difference between the two sides of the membrane, owing to the presence of a permanent "electrical double layer" in that situation. In fact, the system is precisely analogous to a metallic electrode in a solution of one of its salts and the amount of the potential difference is found to be expressed by a similar formula, in which the concentration of the diffusible ions inside the membrane takes the place of the "electrolytic solution tension" of the metal in the formula of Nernst.

Osmotic pressure, as such, plays a part in various physiological phenomena. The volume of animal cells, the turgor of vegetable cells, the reaction of smooth muscle to drugs, the rate of intracellular reactions, the process of secretion, root pressure, the rate of blood flow, the production of lymph, and the absorption of liquid from tissue spaces are discussed briefly in the text.

Certain cells possess the power of regulating the osmotic pressure of their contents, while the higher animals have developed mechanisms for maintaining that of their blood and body fluids at a constant value.

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CHAPTER VII

ELECTROLYTES AND THEIR ACTION

ELECTROLYTIC DISSOCIATION

In the researches of De Vries on plasmolysis (1884, 1885, 1888), it was found that, if sugar in a certain molar concentration was just sufficient to produce a result, a number of other substances, such as mannitol, etc., in the same molar concentration also produced the same effect. On the other hand, another group, sodium chloride, potassium nitrate, etc., produced the effect in a molar concentration which was *lower* than that of sugar. The relative concentrations of the various substances of the latter group which were plasmolytically, *i.e.*, osmotically, equivalent to that of the former group, were expressed in a series of numerical values, the *isotonic coefficients*.

Raoult (1878, etc.) found similar facts in his investigation of the freezing points of the different solutions in question, and they were also expressed in terms of osmotic pressure by van't Hoff (1885). The symbol i will often be met with as expressing the number by which the osmotic pressure of a substance such as cane-sugar must be multiplied in order to obtain that of an equimolar solution of the particular substance in question to which the given value of i refers. The following table given by Philip (1910, p. 132), from the data in the paper by van't Hoff and Reicher (1889), gives a few values of i calculated (I.) from the depression of the freezing point, (II.) from the plasmolytic experiments of De Vries, and (III.) from electrical conductivity. The meaning of the third column will be seen later.

	Gram-equivalents per Litre.	I.	II.	III.
Potassium chloride	0·14	1·82	1·81	1·86
Calcium nitrate	0·18	2·47	2·48	2·46
Magnesium sulphate	0·38	1·20	1·25	1·35
Calcium chloride	0·184	2·67	2·78	2·42
Potassium ferrocyanide	0·356	...	3·09	3·07

Now, when we remember that the osmotic pressure of a solution is in direct proportion to the number of molecules of the solute present in unit volume, we see that, apparently, a smaller number of molecules of the second group of substances produces the same effect as a larger number of the first group. If we make a solution by adding 1 gram-molecule of potassium chloride to 10 litres of water, we find that its osmotic pressure is about 1·8 times that of a solution made with 1 gram-molecule of cane-sugar.

It is quite clear, therefore, that there are more osmotically active elements in the first solution (potassium chloride) than in that of cane-sugar. Since that of the latter solution corresponds to the number of molecules taken, it follows that, in the former case, the number of active "molecules" has somehow increased. In other words, the molecules must have been split up so as to make a larger number.

When the molecules of gases, such as chlorine at a high temperature, are found to be split up into atoms, so that they no longer appear to obey

Avogadro's law, they are said to be "dissociated." In the same way we may speak of the molecules in our solutions with anomalous osmotic pressures as being "dissociated."

But what sort of dissociation are we to suppose that such a salt as potassium chloride undergoes in water? It is plain that hydrolysis into hydrochloric acid and potassium hydroxide is not to be thought of; these cannot exist together in solution. Moreover, such a hypothesis would not explain the phenomena in the case of acids or bases, which behave in the same way as salts. Again, it cannot be potassium and chlorine in their ordinary state, since potassium is immediately converted by water into its hydroxide, and chlorine would be easily detected if present. Looking at the lists of substances in the two classes referred to, we are at once struck by the fact that those substances which give an abnormally high osmotic pressure and are, as we have seen, "dissociated" in solution in water, are all good conductors of electricity, whereas the "normal" ones are non-conductors. Further, it is found that if a substance which gives an anomalous osmotic pressure in water and is a good conductor is dissolved in a solvent in which it no longer conducts electricity, as, for example, hydrochloric acid in benzene, its osmotic pressure is normal, or, at any rate, not greater than normal.

Suppose that we take a solution of hydrochloric acid in water and pass an electric current through it, we find free chlorine is separated at the anode, where the current enters, and free hydrogen at the cathode, where the current leaves the solution. One of the constituents of the solute, which is called an "electrolyte" when it conducts electricity, "wanders" in one direction, the other in the other direction. Faraday was the first to use the name "electrolyte," and he showed that this is actually the way in which the electric current is carried through the solution of a substance which is capable of conducting it. Each constituent of the electrolyte carries a definite quantity of electricity; in our case, the hydrogen carries positive electricity from the anode to the cathode, and the chlorine carries negative electricity from the cathode to the anode. The name used by Faraday (1834, pp. 78 and 79, and 1839, I. pp. 197, 198) for these electrically charged atoms, or molecules, was "ions" (ἰών, participle of εἶμι, "going"), those carrying a positive charge, which they give up at the cathode, being "cations" (κατά = down), and those with a negative charge "anions" (ἀνά = up), in accordance with the direction in which they move in relation to the current, regarded as of positive electricity. The electrodes are "anode" and "cathode" (ὁδός = way). A portrait of Faraday will be found in Fig. 51. In order to conduct a current, then, the solute must be decomposed into positively and negatively charged parts, that is, "*electrolytically dissociated.*"

We may note here that, according to Nernst (1911, p. 356), the word "ionisation," sometimes used, is better reserved for the case of the gas ions, which are produced by X-rays, ultra-violet light, etc., and consist of a number of molecules of the gas grouped around a single electron.

It is obvious from the facts of electrolytic conduction that hydrogen and chlorine are capable of existence in forms which have quite different properties from those which they possess in their ordinary familiar forms. While they are engaged in carrying electric charges through the solution in which a current passes, they cannot be recognised as hydrogen and chlorine.

The actual amount of electricity carried by a univalent ion is, as was shown by Faraday's work, a definite quantity, and is now known by the name suggested by Johnstone Stoney as an "electron." A bivalent ion carries two electrons and so on. Helmholtz (1881) put forward the view that electricity itself has an atomistic structure, so that, in a certain sense, we may look upon positive and negative electrons as two new univalent elements. Thus a positive electron may be said to replace Cl in HCl, forming hydrogen ion instead of hydrogen chloride (Nernst, 1911, p. 395). If this be so, it is not surprising that hydrogen ion is completely different from hydrogen itself, since it is a new chemical compound, and the essence of chemical combination consists in the manifestation of properties unlike those of the constituents.

The modern development of the science of electrons does not belong to the subject of this book; those interested may consult the short work by Ramsay (1912) on "Elements and Electrons." It is well, however, to refer to one point. The existence of two kinds of electrons, positive and negative, has been assumed above. The question is not yet definitely decided as to whether there is only one kind, the negative, and whether an apparent positive charge is really only the absence of a negative one. This does not affect the argument, and there is evidence in favour of the existence of positive electricity.

Since a hydrogen atom is a very different thing from a hydrogen ion it is not permissible to use the same symbol for both. It is generally agreed to use a dot for a single positive charge and a dash for a single negative one, repeating them as many times as the valency of the ion requires. Thus, $H^{\cdot}$, $Ca^{\cdot\cdot}$, $NH_4^{\cdot}$ are positive ions, and Cl', SO_4'', PO_4''' are negative ions. The signs + and −, used at one time, are no doubt more expressive, but cause difficulties to the printer, when added to the top of a symbol and, in other positions, would be liable to cause confusion.

Although it is most convenient to speak of the possession of positive and negative charges, it should be remembered that it is possible that the apparent presence of a positive charge may mean simply the absence of a negative one, so that, for example, $H^{\cdot}$ means that the hydrogen ion has one less negative electron than an "uncharged" atom and two less than OH'.

FIG. 51. PORTRAIT OF MICHAEL FARADAY.

So far we have spoken only of the ions present in a solution through which an electrical current is actually passing. Now Clausius (1857) pointed out that, in order to explain the phenomena of electrolysis, a part of the molecules of the electrolyte must be assumed to be *already* dissociated into ions, which possess movements independent of one another. In the case of the solutions with anomalous osmotic pressures we notice, in the table given on page 169 above, that the "isotonic coefficient," or van't Hoff's factor i, that is the ratio between the actual osmotic pressure of a solution of an electrolyte, and that which it would have if it contained only non-dissociated molecules, is not a whole number, although in dilute solutions of strong acids and bases it is very near being so. In dilute hydrochloric acid it is practically 2, but in sodium chloride of 0·1 per cent. it is only 1·9. Measurements of electrical conductivity show the same ratio between the part of the solute that carries the current, *i.e.*, the ions, and the non-dissociated fraction which takes no part in the process, as the table referred to shows.

Arrhenius (1887), on considering these various facts, was led to see that the anomalous osmotic pressures of solutions of electrolytes could be very simply explained by the assumption that the dissociation into ions is not merely the state during the passage of a current, but is the normal condition of the solution of an

electrolyte under any circumstances. Evidence that this is so will be given presently. The theory is that known as the "electrolytic dissociation theory,"

FIG. 52. PORTRAIT OF ARRHENIUS.

(From a photograph by A. Jonason, Göteborg.)

which plays so large a part in science at the present day. A portrait of Arrhenius is given in Fig. 52.

Arrhenius had already suggested naming those molecules which take part in the passage of an electrical current, and whose ions are independent of one another in their movements, "active," and those whose ions are firmly combined together "inactive," and in his classical paper, "Ueber die Dissociation der im Wasser

gelöster Stoffe" (1887, p. 637), he gives the evidence for the acceptance of two hypotheses, which are:—

"1. The Law of van't Hoff applies not only to the greater number of substances, but to all, including those which had been considered to be exceptions (electrolytes in watery solution)."

This law of van't Hoff, which is a generalisation of Avogadro's law, has already been quoted (page 148 above), but, for reference, it may be repeated here:—

"The pressure which a gas possesses at a given temperature, when a definite number of molecules are present in a definite volume, is of the same value as the osmotic pressure which is exerted, under the same conditions, by the greater number of substances, when they are dissolved in any kind of liquid."

The second hypothesis of Arrhenius states that:—

"2. All electrolytes (in solution in water) consist partly of molecules which are active (in electrolytic and chemical relationships), and partly of inactive molecules. The latter, however, on dilution, are converted into active molecules; so that in infinitely diluted solutions only active molecules are present."

We may now venture to call these hypotheses "laws," although objections have not been wanting, as we shall see.

Free ions, therefore, are believed to be present in all solutions of electrolytes, whether a current is passing or not. Definite proof of their existence under ordinary conditions is naturally desirable. Since the distinguishing property of an ion is its electrical charge, the difficulty of investigating its properties by methods other than electrical, which might be supposed to introduce conditions which beg the question, is obvious. To begin with, the following reasons are given by J. J. Thomson (1888, p. 294) for concluding "that the splitting up of the molecules which allows the current to pass is not caused by the electro-motive force, but takes place quite independently of the electric field." In other words, they are already split up before the current is sent in.

(1) The smallest electro-motive force is sufficient to start a current, so that no finite electro-motive force is required to dissociate the molecules.

(2) The experiments of Fitzgerald and Trouton (1886, p. 312) show that Ohm's law is obeyed exactly, "whereas, if the electro-motive force had to break up the molecules, the current would be proportional to a higher power than the first of the electro-motive force."

(3) J. J. Thomson himself was unable to detect the slightest change in the osmotic pressure of a solution of an electrolyte during passage of a current through it. If the number of separate systems is increased by the current, the osmotic pressure must rise considerably; in the case of dilute strong acids, to nearly double.

Let us further contrast the behaviour of an organic compound of chlorine, say chloroform, $CHCl_3$, with that of an inorganic chloride, $CaCl_2$. In the first case, chlorine cannot be detected by the ordinary tests; the molecule has certain properties as a whole. In the second case, in dilute solution, the behaviour to reagents is not that of an individual compound with its own peculiar properties; its reactions are simply those which are common to all calcium salts, together with those which are common to all chlorides. According to the electrolytic dissociation theory, all chlorides, in dilute solution, contain chlorine ions, and all calcium salts contain calcium ions. Calcium salts have also a particular action on the muscle of the heart, and it is found that it does not matter what salt is taken. Again, hydrochloric acid has no properties peculiar to itself; it tastes sour, turns litmus red, dissolves metals, inverts cane-sugar, in common with all acids. It precipitates silver salts in common with all chlorides. The nitrate, chloride, and bromide of copper are all blue in dilute solution in water, but in alcohol, where very little dissociation is to be expected, they are blue, green, and brown respectively. Ostwald (1892) has shown that each ion independently contributes its share to the properties of a solution, inclusive of colour, by taking photographs of the absorption spectra of the permanganates of zinc, cadmium, ammonium, tin, potassium, nickel, magnesium, copper, hydrogen, aluminium, sodium, barium, and cobalt in dilute

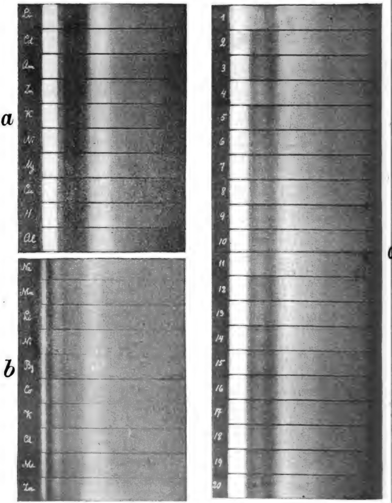

FIG. 53. ABSORPTION SPECTRA OF SOLUTIONS CONTAINING THE SAME COLOURED ION.—Showing the identity of position of the band.

a, Permanganates of the elements given at the side.
b, Salts of diazoresorufin with various elements.
c, Salts of rosaniline with the following acids:—

1. Laevulinic.
2. Acetic.
3. Chloric.
4. Benzoic.
5. Hydrochloric.
6. Sulphanilic.
7. Nitric.
8. Phthalamidoacetic.
9. Butyric.
10. Phenylpropiolic.
11. Hyposulphurous.
12. Trichlorlactic.
13. Glycollic.
14. Phthalanilic.
15. Perchloric.
16. Salicylic.
17. Monochloracetic.
18. Lactic.
19. Orthonitrobenzoic.
20. Sulphuric.

(After Ostwald.)

solution, and found the absorption band in the same situation in all. Various salts of dyes show the same behaviour (see Fig. 53).

Before we pass on to consider other evidence, the question of electrolytic conductivity must be dealt with.

ELECTROLYTIC CONDUCTIVITY

The passage of an electric current through a solution being due to the ions present between the electrodes, it is clear that the amount of current that will pass through a given solution will depend, in the first place, on the size of the electrodes—the larger they are, the more ions there will be between them. The current that passes, other things being equal, is in direct proportion to the area of the electrodes when the column of solution between them is of the same cross section as the electrodes, so that no spreading of the current takes place. In the second place, owing to the fact that the velocity with which the ions move is finite, the greater the distance between the electrodes the longer it will take for an ion to carry its charge to the opposite electrode and the less electricity will be carried in unit time, *i.e.*, the current will be less. In comparing the conductivity of one solution with that of another, it is therefore necessary to agree to some arbitrary dimensions. The unit of conductivity is taken, accordingly, as that of a body of which a column one centimetre long and one square centimetre in cross section has a resistance of one ohm (Nernst, 1911, p. 361). The resistance is the reciprocal of the conductivity; if one solution has twice the resistance of another, only half the current will pass through it, so that its conductivity is half that of the other.

If, then, a body of the dimensions given above has a resistance w in ohms (usually written ω) its conductivity (κ) is $\frac{1}{w}$ in reciprocal ohms, frequently called mhos (*i.e.*, ohm spelt backwards). The actual conductivity of a particular solution is called the "*specific conductivity*" of that solution; but in order to compare solutions of different salts with one another, it is convenient to have an expression in which the molar concentration is taken into account. The "*equivalent conductivity*" is now understood as the actual conductivity divided by the concentration in gram-equivalents per cubic centimetre (η), *i.e.*, $\frac{\kappa}{\eta}$, and is denoted by Λ. It is clear that the conductivity of the solution of an electrolyte depends on its concentration, since it is the ions into which the solute dissociates that conduct the current, and the more there are in the space between the electrodes, the more current will pass. The value of taking gram-equivalents instead of gram-molecules is that salts with multivalent ions are more readily compared with those with univalent ions. Thus, if equimolar solutions of KCl and K_2SO_4 are compared, we must remember that the second salt, at an equal degree of dissociation, has twice the conducting power of the first, since it gives ions with four charges, two negative and two positive, while the first only gives one negative and one positive.

It will be remembered that, in the statement of the theory of electrolytic dissociation given by Arrhenius (page 173 above), the "inactive molecules" are said to be converted into active molecules on dilution. This is the expression of the experimental fact that the equivalent conductivity, or the number of ions into which a gram-equivalent is dissociated, increases as the solution is diluted. By plotting successive values of the equivalent conductivity at increasing dilutions in the form of a curve, the value at infinite dilution, that is, what it would be if completely dissociated, can be extrapolated. Equivalent conductivity may also be expressed in terms of the volume of solution in cubic centimetres which contains 1 gram-equivalent; the symbol ϕ is generally used, so that the equivalent conductivity may be expressed as $\kappa\phi$. ϕ is, of course, equal to $\frac{1}{\eta}$.

The *methods of measuring conductivity* may be now considered. What is actually measured is the resistance of a stratum of known dimensions. The value

of these dimensions for a particular vessel is determined by measuring in it the resistance of a solution whose value in conductivity is known from previous measurements in a vessel whose dimensions can be measured directly. Actual details may be obtained from the book by Findlay (1906, pp. 144-181) or from the article by Asher (1911, pp. 161-174). In the present pages general principles only need be referred to. As is familiar to the reader, the measurement of the resistance of metallic conductors by the Wheatstone bridge method is capable of extreme accuracy. The same method, with modifications, is also employed for solutions of electrolytes. These modifications are due to the fact that, if a current is sent for any appreciable length of time between metallic electrodes immersed in such a solution, the current falls off greatly in strength owing to deposition of ions on each electrode of opposite sign to themselves, *polarisation*, as it is called. For this reason, accurate measurements by the ordinary galvanometer method are impossible. The difficulty is got over in the method of Kohlrausch by the use of a current which rapidly changes its direction, before any appreciable polarisation has had time to develop. Each electrode is made anode and cathode in turn. A small induction coil, with a very rapidly vibrating interrupter, is used for the purpose and the alternating induced currents from the secondary coil are sent through the electrolyte. But this again necessitates the use, as detector of the zero point, of some instrument which responds to alternating currents, since the ordinary galvanometer does not, except when the changes of direction do not occur at frequent intervals. A telephone is generally used.

When the solutions have a very high resistance, it is found to be difficult to get enough current through to give sharp readings with the telephone. In such cases, the method of Whetham (1900) is of great value. In this, the change of direction of the current is effected by a rotating commutator, and, in order to enable a delicate galvanometer of the ordinary type to be used, the alternating current is rectified again before going to the galvanometer. This is done by a second commutator on the same axis as that which originally makes the alternating current. This method allows of great variations in the electromotive force used to drive the current through the electrolyte, and in the sensibility of the galvanometer. It seems probable that a thermionic valve might be used to rectify the alternating currents from an induction coil and thus enable a galvanometer to be used as detector. The most convenient vessels for physiological use are those of Henry (Fig. 21A of Asher's article, 1911).

We may now return to the consideration of further evidence in favour of the electrolytic dissociation theory.

IONIC CONDUCTIVITY

Let us take the molecular conductivity of the following series of salts in 0·0001 molar concentration as given by Kohlrausch and Maltby (1899).

	Chloride.	Nitrate.
K	129·05	125·49
Na	108·06	104·53
Li	98·06	94·38

The precise units in which these are expressed does not matter for our present purpose, since all are in the same units.

The difference between KCl and NaCl is 20·99 and between KNO_3 and $NaNO_3$ is 20·96, practically identical. Again, the difference between KCl and LiCl is 30·99 and between KNO_3 and $LiNO_3$ is 31·11. What does this imply? Obviously that it does not matter whether, in changing Na or Li for K, we take a chloride or a nitrate; that is, the metallic part of the salt makes a certain contribution to the conductivity which is independent of the acidic radical associated with it. Similarly, the difference between KCl and KNO_3 is 3·56, between NaCl and $NaNO_3$ 3·53, and between LiCl and $LiNO_3$ 3·68, so that the same consideration applies to the other radical. This fact may perhaps be clearer if put in a symbolic form:

$$(K + Cl) - (Na + Cl) = (K + NO_3) - (Na + NO_3)$$

$$\text{and} \quad (K + Cl) - (K + NO_3) = (Na + Cl) - (Na + NO_3)$$

can only hold, if K, Na, Cl and NO_3 each has a definite value independent of that of any of the others.

We may conclude, then, that the conductivity of a highly diluted solution is made up of the independent conductivities of the individual ions and, if this is so, these ions must be present as separate entities. Kohlrausch expresses this in what is generally known as his *law of the independent migration of the ions.* The symbol u is given to the part contributed by the cation and v to that contributed by the anion, so that the molecular conductivity at infinite dilution of a binary electrolyte (that is, one that dissociates into two univalent ions) is $u+v$. These are constant values for the same ions, whatever salts they may form constituents of.

Referring back to the table on page 176, we notice that the conductivity of the Li ion is less than that of the Na ion and this again is less than that of the K ion. Since each of these ions carries the same charge, it follows that they must travel at different rates. A little consideration will show that, if this be so, after electrolysis by passage of a current has gone on for some time, there will be a different concentration of the electrolyte around the two electrodes and, by this means, measurements of the rates of the various ions have been made by Hittorf. Details of these measurements will be found in the textbooks (Philip, 1910, pp. 143, etc.; Nernst, 1911, pp. 362, etc.).

The following table gives the molecular conductivities of a number of ions at the temperature of 18° (Nernst, 1911, p. 366).

K	NH_4	Na	Li	Ag	H		
$u=65{\cdot}3$	64·2	44·4	35·5	55·7	318		
Cl	Br	I	NO_3	ClO_3	CO_2H	$C_2H_3O_2$	OH
$v=65{\cdot}9$	66·7	66·7	60·8	56·5	45	33·7	174

In the case of large organic ions it is interesting to note that the rate of migration diminishes comparatively little with increasing size. Thus, according to Bredig (1894), at 25°, the values of certain anions are as follows:—

Anion of	Number of Atoms.	Rate of Movement.	Conductivity of Na Salt, Infinite Dilution.
Acetic acid	7	38·3	87·5
Caproic acid	19	27·4	76·6
Picric acid	18	31·5	80·7
Lactone of o-Toluido-β-isobutyric acid	40	23·0	72·2

The practical use of these facts is that we can calculate the values of the molecular conductivity at infinite dilution in cases where it cannot be obtained experimentally. Thus ammonium hydroxide, even when diluted so far that the accuracy of the measurements becomes uncertain, is a considerable distance from complete dissociation. But from the law of Kohlrausch we can obtain the value as the sum of those of the constituents, $NH_4^{\cdot}$ and OH', viz.:—

$$64{\cdot}2+174=238{\cdot}2.$$

Knowing the conductivity of salts when completely dissociated, we can thus determine the *degree of dissociation* at any concentration from measurements of its conductivity at that concentration. Suppose that we find that a binary salt at a known concentration has a molecular conductivity half that which we obtain from Kohlrausch's law as the limiting value at infinite dilution, we know that only half of its molecules are taking part in the conduction of the current.

The *actual rate of movement* of the various ions is of some interest. As Nernst points out (1911, p. 363), the small dimensions of ions would lead us to expect that the frictional resistance to their movements is very great. Their velocity is therefore proportional to the force acting on them. If the fall of potential in the solution is 1 volt per centimetre, that is, if the electrodes are 10 cm. apart and a potential difference of 10 volts exists between them, the hydrogen ion moves at the rate of 0·0033 cm. per second and the potassium ion at 0·00067 cm. per second. The actual manner in which this is determined is beyond the scope of this book.

This slow movement of ions allows another piece of evidence to be brought in favour of the actual existence of ions in solutions of electrolytes apart from any passage of current through them. Take a solution of copper sulphate and place in it two electrodes at 2·2 cm apart. Let the anode consist of copper and the cathode of platinum. As soon as the current is established, copper is deposited on the platinum plate and dissolved from the anode by the SO_4'' ion. Now, if the electrical current itself split up the $CuSO_4$ molecules and the two oppositely charged parts were attracted to the two opposite poles, according to the old view, it follows that the SO_4'' ion belonging to a particular copper ion at the cathode has to travel in our case 2·2 cm. in less than one second. Suppose that the potential difference were 2·2 volts and that we ascribe to the SO_4'' ion as great a velocity as that of the OH' ion (0·0018 cm. per second) (it is really much less), twenty minutes will be required for it to travel the distance of 2·2 cm. between the electrodes.

Ostwald (1888, p. 272) directs attention to another similar experiment. It is well known that, if amalgamated zinc be immersed in dilute sulphuric acid, it is not attacked. But if a piece of platinum be also immersed in the same solution, even at a considerable distance, as soon as the two metals are connected by a wire, hydrogen appears on the platinum and zinc goes into solution. The hydrogen cannot arise from the same sulphuric acid molecule whose SO_4 attacks the zinc, since it cannot travel the distance in the time. It must come from the immediate neighbourhood and have been already present as dissociated ionic hydrogen.

Another fact which is readily explained by the different rate of migration of ions already present and for which no other explanation is at hand, is that, when a solution of an electrolyte is in contact with water, a potential difference is nearly always found to exist at the boundary surface. This is due to the unequal rate of diffusion of the two ions, so that either the anion or the cation is in advance of the other, forming a Helmholtz double layer. Of course, they cannot separate far from one another, on account of electrostatic attraction. We shall meet with this phenomenon again in connection with the sources of electrical changes in living tissues.

HYDRATION OF IONS

When we look at the numbers in the table of ionic conductivities on page 177 above, we are struck by the fact that lithium, with an atomic weight of 7, moves at a much slower rate than potassium, with an atomic weight of 39. The explanation is probably that the lithium ion carries with it a larger number of water molecules than the potassium ion, so that greater friction is experienced.

The chief work on this question has been done by Bousfield (1905, 1906, 1912), to whose papers the reader is referred. An interesting fact, which is worth quoting, comes out from the results of the last paper (1912, p. 168). The number of molecules of water combined with both ions at infinite dilution is for

KCl	NaCl	LiCl
9	13	21

There are reasons for supposing that the number combined with the Cl' ion is 5, since its transport number is just a little greater than that of potassium, so that its share must be a little more than half of the total 9 of KCl. If this is so, we have for the number of molecules of water associated with the ions of

K	Na	Li
4	8	16

As the author says, "an attractive looking series."

FURTHER EVIDENCE AND SOME DIFFICULTIES

The chief evidence for the truth of the electrolytic dissociation theory is, undoubtedly, the fact that it is capable of giving correct *quantitative* explanation of so many phenomena, and even of predicting the numerical values of the factors in these phenomena. It is not surprising that deductions from it have not always been verified, since modifications and additions are always necessary in theories of such far-reaching application.

Objections have been brought against it, but no rival theory has been shown able to afford the accurate quantitative results that it does in so simple and direct a manner. At the present time it may safely be said to be indispensable. There are many phenomena which, without it, could not even be described except with difficulty, much less treated quantitatively. Of these we shall presently meet with some striking examples.

One only need be mentioned now. As we shall see, the "acidity" of a solution is readily expressed on our theory by the number expressing its concentration in $H^{\cdot}$ ions. The difficulty found by those who do not accept the theory is seen on p. 576 of the paper by E. F. and H. E. Armstrong (1913), where mixtures of acid and alkaline phosphates in certain proportions have to be used, giving a set of numbers, having only a meaning relative to one another.

Some of the *difficulties* may be referred to, chiefly for the purpose of keeping in mind where further research is needed, but also on account of their instructive nature.

At the time of the first publication of the theory, objection was made to it on the ground that, in the case of ammonium chloride, it was possible to separate by diffusion the products of dissociation, NH_3 and HCl, whereas this could not be done in the case of Na and Cl ions in water. Although, at the present time, the explanation given by Arrhenius (1901, p. 176) is generally accepted, it is instructive to refer to it on account of the fact that it turns up in various forms. This explanation rests on the existence of the electric charge on the ions, whereas the products of ordinary dissociation are devoid of charge. This charge is the very large one of 96,500 coulombs per equivalent. Suppose, then, that we have in a tube a stratum of water lying over one of a solution of sodium chloride. If the Na and Cl had no charge, the latter, which diffuses much more rapidly than Na (in the ratio of 68 to 45), would be found in excess in the water layer after a short time. But when only 10^{-13} gram-equivalents of Cl in excess of Na ions have passed to the upper layer, this layer would have a negative charge of $96{,}500 \times 10^{-13}$ coulombs or $96{,}500 \times 10^{-13} \times 3 \times 10^{10} = 290$ electrostatic units, a quantity of electricity which would, on a sphere of 10 cm. radius, give a spark of 0·3 cm. Now it is easy to calculate that the electrical forces produced by the undetectable amount of 10^{-13} gram-equivalents of Cl far exceed any possible osmotic force which would cause unequal diffusion of the two ions. The electrostatic unit of electromotive force is about 300 volts, so that the above-mentioned 290 units would give a potential of $\frac{290 \times 300}{10} = 8{,}700$ volts, on a sphere of 10 cm. radius, in round numbers say 10^4 volts. This would be about the same if the charge were given to a cube of liquid of 10 cm. side in a diffusion vessel.

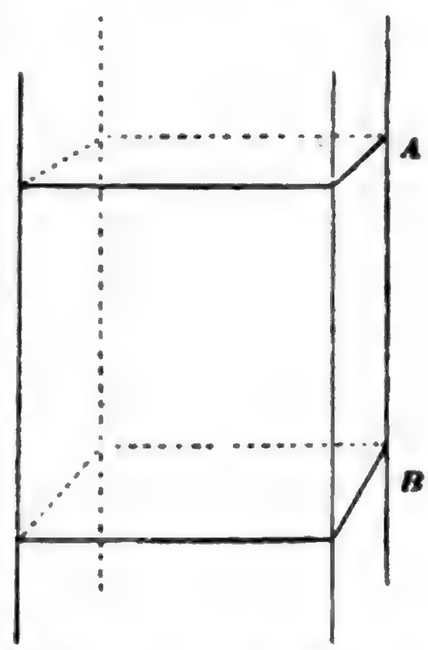

FIG. 54. DIAGRAM FOR CALCULATION OF THE ELECTROSTATIC ATTRACTION BETWEEN OPPOSITELY CHARGED IONS.

(Arrhenius.)

Let us take now a stratum of half normal sodium chloride solution one centimetre high and one square centimetre in section, and imagine a potential of 10^4 volts at the end A and zero potential at B (Fig. 54).

The sodium chloride is further supposed to be distributed in such a manner that its concentration at A is zero and at B normal, half normal midway. It is assumed to be completely dissociated for sake of simplicity. On the Cl ions there is acting an electrical force of $\frac{V}{l} \times e$, where $\frac{V}{l}$ is the fall of potential per centimetre, *i.e.*, 10^4 volts, and e is the amount of charge on the ions, *i.e.*, $\frac{96{,}500}{2000} = 48{\cdot}2$ coulombs, since the solution contains per cubic centimetre $\frac{0{\cdot}5}{1000}$ gram ions. The total force acting is—

$48{\cdot}2 \times 10^4$-volt-coulombs per centimetre (Arrhenius, 1901, p. 6) $= 48{\cdot}2 \times 10^{11}$ dynes. The osmotic force, on the other hand, which acts on the same Cl ions is given by the difference between the osmotic pressures of the normal solution at B and that of zero concentration at A, *i.e.*, at 18°, $22{\cdot}4 \times \frac{273 + 18}{273} = 24{\cdot}2$ atmospheres, or

$23{\cdot}9 \times 10^6$ dynes. The osmotic force is therefore 2×10^5 times less than the electrostatic force preventing diffusion; in other words, the latter is 200,000 times as strong. We see then how an extraordinarily minute excess of Cl over Na ions would suffice to prevent any further diffusion. If one ion moves on, the opposite one must follow it at an infinitesimal distance.

A more difficult question arises from considerations of energetics. We know that sodium and chlorine combine with the evolution of heat in considerable amount, so that, in order to separate them as ions when the compound is dissolved in water, a corresponding amount of energy must be supplied from some source. The fact that ions are hydrated seems to offer a possibility, if we regard the hydrates as chemical compounds, formed with evolution of heat.

Bousfield and Lowry (1907, p. 125) suggested that the affinity of the "ionic nucleus" for water is the main source of the energy required. Further evidence for this view is given by Bousfield (1912, p. 149). The argument may be put very briefly thus: Atoms, being composed of large numbers of more minute bodies or corpuscles, may be regarded as compressible. The heat of formation of a compound is found to be approximately equal to the sum of certain "calorific constants" of the components, together with $0{\cdot}875\ \delta V$, where δV is the change of atomic volume which takes place. From this fact, the internal energy of an atom is to be regarded as the sum of the kinetic energy of the corpuscles, and of the potential energy due to their mutual attraction. The factor, $0{\cdot}875\ \delta V$, therefore represents the change in internal energy of the atoms due to their change of volume on combination. How compression or contraction diminishes the internal energy of an atom by approximation of mutually attractive corpuscles may be found on p. 151 of the original paper. Applying these considerations to the estimation of the components of the heat of formation of solid, liquid, and ionic molecules, it is found that δV is considerably greater in the ionic state than in that of solid or liquid; thus, the value for KCl in the ionic state is 42·7, for the solid state, 32·5. That is, the contraction which takes place on combination in the ionic state is greater than that in the solid state, and may well be the source of the energy required for electrolytic dissociation.

Larmor (1908, p. 37) states the possibility that "internal potential energy is released owing to the ions entering into relations of closer affinity with the solvent." There is, of course, no doubt as to the capacity of molecular forces to afford the energy required, but the question still remains, what should cause them to give it up for the purpose of dissociating dissolved salts? We may say that the affinity of an ion for water is greater than that which it has for an opposite ion, but is this any more than a re-statement of the problem in another form?

A further difficulty that has been put forward is this. Granting that, by some means or other, the ions have been separated, what is to prevent the opposite electrical charges from neutralising one another with production of the salt again? We have seen that an analogous process does actually occur in the mutual precipitation of oppositely charged colloids. The answer is probably bound up with that to the previous question. The forces which caused the dissociation are presumably continually active in preventing recombination.

There is no doubt that the *dielectric constant* of the solvent is, in some way, intimately involved in the process. It is not an easy matter to picture the way in which it acts, but the following points may possibly be of assistance to the reader.

Two oppositely charged bodies, as is well known, attract one another with a certain force, which can be measured. It might be supposed that this force would be independent of the substance between the two bodies, provided that it be an insulator. But this is not so, as Faraday found. Suppose that air is the insulator between the two bodies, and that they have such a charge, and are at such a distance from one another, that the force tending to bring them together is equal to the weight of 10 g. Now put petroleum in place of air, it is found that the force is only 10/2·2, and, if castor oil be used, it is only 10/4·3. The denominators of these fractions are known as the "dielectric constants" of the liquids, and they play a part in other connections. The capacity of a condenser, for instance, is greater, the greater the dielectric constant of the material between the plates; that of mica being 8, the reason for using this material instead of paraffin, with a dielectric constant of only 2·3, is obvious. According to the modern theory of electrons, the dielectric constant is the greater, the larger the number of electrons present in a given space of the substance. These act as conducting particles and are surrounded by an insulating substance of very special properties, the luminiferous ether. In the course of their propagation through a non-conductor, electric forces must exert an action on these electrons; so that it can be understood why, the more of them there are, the greater is the obstruction to the forces. The connection between electricity and light, as the reader will remember, was worked out by Clerk Maxwell, and, in the present connection, it is of interest to recall the fact that the dielectric constant of a substance is identical with the square of its refractive index, as calculated for light of very long wave length or electric waves (*Maxwell's Law*).

Of all liquids, with the exception of prussic acid, hydrogen peroxide, and formamide, water has the highest dielectric constant, about 80 times that of air, while the majority of other liquids have values which vary between 40 for nitromethane, and 6·46 for acetic acid. When a substance is soluble in more than one of these various liquids, it is found that its conductivity, or, in other words, the degree to which it is dissociated, is greater, the higher the dielectric constant of the solvent (J. J. Thomson, 1893, and Nernst, 1894, independently). The following numbers will serve as illustrations (Walden, 1906). The solute is tetra-ethyl-ammonium iodide, on account of its solubility in a variety of organic solvents.

Dielectric Constant.	Solvent.	Degree of Dissociation in Dilution of 100 Litres =0·01 Molar.
84	Formamide	93 per cent.
81·7	Water	91 ,,
40	Nitromethane	78 ,,
21	Acetone	50 ,,
14	Salicylic aldehyde	34 ,,

Centnerszwer (1902, p. 223) gives the molecular conductivity of potassium iodide in prussic acid as 262, compared with that in water as 80. The dielectric constant of liquid prussic acid is 95.

The significance of this fact in connection with the meaning of the dielectric constant as allowing charged bodies to approach nearer to one another without union of their charges is that, supposing we assume that the oppositely charged ions have been separated, a solvent with a high dielectric constant will enable them to come much nearer to one another without combination than in a solvent with a low dielectric constant. The kinetic energy they possess enables them to resist the attraction of the opposite ions when much nearer together, owing to this attractive force being less the higher the dielectric constant of the solvent; so that, on an average, a larger number are free at any given moment.

Although considerations of such a kind enable us to form some idea of the reasons why ions do not all combine with their oppositely charged fellows, it is not obvious what causes their original separation, when a solid salt is placed in water. If we admit Faraday's view of the electrical nature of chemical affinity, it seems possible that the electronic forces of the dielectric may be involved. When molecules are separated from one another, as in the process of dissolving a solid, it may be that they are more accessible to forces tending to break the combination between their constituent ions, and as the separation is effected, the high insulating power, or dielectric constant, of the solvent prevents, to a varying degree, their recombination.

Perhaps the most serious difficulty in the Arrhenius theory is the behaviour of strong acids, strong bases and salts, as compared with that of weak acids and weak bases. In the latter case, as Ostwald showed, the proportion of dissociated to combined molecules, when the solution is diluted, obeys a law deduced from mass action simply and known as Ostwald's "*dilution law.*" In the former case the law is quite different. In a paper by A. A. Noyes, Melcher, Cooper, and Eastman (1910, p. 375), attention is called to the fact that the electrolytic dissociation in the former case of salts, strong acids, and strong bases "is a phenomenon primarily determined not by specific chemical affinities, but by electrical forces arising from the charges on the ions; that it is not effected (except in a secondary degree) by chemical mass action, but is regulated by certain general, comparatively simple, laws, fairly well established empirically, but of unknown theoretical significance; and that, therefore, it is a phenomenon quite distinct in almost all its respects from the phenomenon of dissociation ordinarily exhibited by chemical substances, including that of the ionisation of *weak* acids and bases."

The reasons for this view can be found in the original paper; we must be content here with reference to the similar dissociation values for salts of different chemical nature but of

the same ionic type, the proportion of these values to valency, the small effect of temperature on the dissociation of salts, strong acids and bases, and its parallelism with that on the dielectric constant, the exponential relation between dissociation and concentration, which is not the same as that required by the law of mass action, and the fact that the optical and similar properties of dissociated salts (in equimolar concentration) is independent of this actual concentration, and therefore of their dissociation, if the solution is even moderately dilute.

With respect to the influence of *temperature*, the actual effect on dissociation must be distinguished from that on the rate of migration of the ions. The temperature coefficient of conductivity of a salt is about 2 per cent. per degree, as shown by Arrhenius (1901, p. 136), but this is almost entirely accounted for by the increased velocity of the ions, due to diminution of internal friction of the solvent. The actual increase in number of ions is very small indeed. In another class of cases, which are regarded by Noyes and his co-workers (1910) as being of a more strictly chemical nature (see below), the increase in number of ions is considerable as the temperature is raised. Water itself is a striking example. According to the data of Kohlrausch and Heydweiller (1894, p. 209), the temperature coefficient of *ionisation* of water at 18° is 5·32 per cent. (Nernst, 1911, p. 670). This fact is in agreement with the great heat of electrolytic dissociation of water.

As remarked above, Noyes and his coadjutors (1910, p. 376) suggest that ions may form two different kinds of molecules, electrical and chemical. In the first case the union is not so strong, and the constituents still retain their electrical charges and their characteristic optical effects. "Secondarily, the ions may unite in a more intimate way to form ordinary uncharged molecules, whose constituents have completely lost their identity and original characteristics." "In the case of salts, inorganic acids and bases, the tendency to form chemical molecules is comparatively slight, so that the neutral electrical molecules predominate. In the organic acids, as a rule, chemical molecules predominate. These latter are formed in accordance with the law of mass action, while electrical molecules are formed in accordance with an entirely different principle, whose theoretical basis is not understood."

G. N. Lewis (1910, p. 218) also calls attention to the deviation of these salts, strong acids and bases, from the mass action law, and points out that it is the moderately concentrated solutions that are abnormal; in highly dilute solutions the behaviour is in agreement. The ions themselves seem to obey the laws of perfect solutions, so that we must turn to the undissociated molecules for an explanation of the anomalies. The author refers to cases where, assuming normal behaviour of ions, correct results are predicted, although the undissociated part is neglected.

A deduction from the electrolytic dissociation theory, which has been verified by independent methods, is the constancy of the product of the concentrations of $H^{\cdot}$ and OH' ions in dilute aqueous solutions. Finally, the Nernst equation for the electromotive force of concentration batteries gives good results when the concentration of the ions alone is considered. Lewis (p. 219) also refers to a calculation which he made involving the use of three principles all founded on the Arrhenius theory, viz., the Nernst equation, the solubility product, and the dissociation constant of water. The result was different from the value accepted, but independent investigation by Haber and by Nernst immediately afterwards showed perfect agreement with the calculated value. As the author remarks: "The calculation would obviously have been vitiated if any one of the principles used had been unreliable." On the whole, the evidence indicates that later and better theories will be developments of the first simple one of Arrhenius, not substitutes for it. It must not be forgotten that the propounder of the theory has always been ready to admit the difficulties. Whether the views of Noyes will be found to explain some of these remains to be seen; there are no doubt many objections to be made to their bare present form. Perhaps this point of view may also supply an answer to the question why a concentrated solution, say of potassium chloride, in which only 25 per cent. is dissociated, exhibits only the properties of ions. Has the KCl molecule no properties of its own?

Arrhenius himself (1914, p. 1424) points out that the dielectric constant of the solvent is increased by the presence of strong electrolytes of higher dielectric constants than itself. This would increase its dissociating power.

It must be admitted that some intemperate partisans of the electrolytic dissociation theory may have claimed too much; at any rate, the sweeping statement that all chemical reactions are between ions must not be made without the qualification that no absolute proof of the absence of the intervention of electrical forces has been given in any particular reaction.

THE ACTION OF IONS IN PHYSIOLOGICAL PROCESSES

When we come to consider the part which electrolytes play in the processes of the living organism, we have to note that there are three modes in which they may act. In the discussion of the colloidal state, we saw that, in the intervention of neutral salts in such phenomena, we may distinguish, in the first place, an effect connected with the electrical charge on the ions, specially marked with ions of valencies above one, and not in relation to the chemical nature of the ions; so that the effect, say, of $Ca^{\cdot\cdot}$ is not to be distinguished from that of $Ba^{\cdot\cdot}$. Especially in the case of multivalent ions, this action is manifested by very small concentration. It may be illustrated by the effect of simple trivalent ions on the heart, an action which does not seem to be associated with the chemical nature of these ions, since it is shown by a large number of them, and in extraordinarily low concentrations (Mines, 1911).

In the second place, there is an action shown by salts usually in somewhat high concentration, which is not directly connected with their electrical charges as such, and is most satisfactorily explained as being an action of some kind on the solvent, "*lyotropic*," as it is called by Freundlich. This is shown in the "salting out" of proteins, and in the various effects of anions and cations on such processes as imbibition, in which the "Hofmeister series" is followed.

In the third place, there are the actions in which differences of a more chemical kind come into play. Such cases are those of potassium and sodium salts on the heart muscle. In these, we know that it is the ions which are concerned, and not the molecules of the salts, by the facts that the action is shown by solutions so dilute that undissociated molecules are nearly absent, and that it does not matter what particular salts of these metals are used.

Other instances that may be given are the effect of calcium ions on the clotting of blood, in which even closely related elements, such as barium, are unable to replace calcium; and the powerful action of barium in producing contraction of smooth muscle.

HYDROGEN AND HYDROXYL IONS

The great activity of acids and bases in various ways is a familiar fact, so that, in our consideration of the various ions of physiological importance, it is natural to take these first.

It is also a matter of common experience that the properties associated with them are much more strongly marked in the case of certain chemical individuals than in others. Some acids will turn out others from combination; their solutions, in equal strength, taste much sourer, and some invert solutions of cane-sugar more rapidly than others, in the same molar concentration, do.

It is here that the electrolytic dissociation theory has shown itself to be of especial value, in that it is able to give precise numerical values to express the acid or alkaline properties of a solution. Now what, according to this doctrine, is the character common to all acids and what to all bases? Obviously, the hydrogen ion in the first case and the hydroxyl ion in the second. Hydrochloric and acetic acids in solution are dissociated into $H^{\cdot}$ and Cl' and into $H^{\cdot}$ and acetic anion respectively; the only chemical substance common to both is the $H^{\cdot}$ ion. But why is hydrochloric acid the stronger of the two, as is so obvious in many ways? The answer is given by measurements of the electrical conductivity of the two. Hydrochloric acid is a much better conductor; it is therefore more highly dissociated and contains a much higher concentration of hydrogen ions. Here we

have, then, a numerical value for the acidity, namely, the concentration in $H^{\cdot}$ ions. Similar considerations apply to bases, say sodium or ammonium hydroxides, and here the concentration in OH' ions gives a measure of the alkalinity of a solution. As will be shown later, the product of the $H^{\cdot}$ and OH' ion concentrations in solutions in water is a constant quantity; it is clear, therefore, that along with any OH' ion concentration a definite $H^{\cdot}$ ion concentration is connected. For the sake of uniformity it is the custom to express both acidity and alkalinity in terms of $H^{\cdot}$ concentration. Thus, neutrality means the concentration of the two ions as they are present in pure water, *i.e.*, 1×10^{-7} at 25°, and any concentration of hydrogen ion less than this means alkalinity and any greater means acidity.

It is sometimes troublesome to refer repeatedly to expressions such as $1{\cdot}3 \times 10^{-6}$ and so on. Hence Sörensen (1909, p. 28) has advocated the use of the negative exponent of 10 as a positive whole number, calling it the "hydrogen-ion-exponent" or $P_{H^{\cdot}}$. Thus 5×10^{-6} is the same as $10^{-5{\cdot}3}$, and a solution having this concentration in hydrogen ions is said to have a $P_{H^{\cdot}}$ of 5·3. A centinormal solution of hydrochloric acid is 0·00916 normal in hydrogen ions. To express this as a power of 10, we must remember that it is the custom, for the convenience of tables, to consider the decimal part of a negative logarithm as positive. Thus log 0·00916 is expressed as $\bar{3}{\cdot}962$ or $-3+0{\cdot}962$, that is, $-2{\cdot}038$, which is the exponent we require, and the $P_{H^{\cdot}}$ is 2·038. This method, although convenient in practice, has certain disadvantages for the beginner. The $P_{H^{\cdot}}$ values *diminish* as the acidity *increases*. Moreover, while it is easy to see that a $H^{\cdot}$ concentration of 4×10^{-6} is double that of 2×10^{-6}, it is not at once obvious that a $P_{H^{\cdot}}$ of 5·398 means double the acidity of one of 5·699. One has to get accustomed to thinking in negative logarithms.

Perhaps one of the most striking facts with regard to acids, and in itself strong evidence of the truth of the Arrhenius theory, is that the heat produced by the neutralisation of equivalent amounts of the most various acids is practically identical. This is easily accounted for if due to the union of the $H^{\cdot}$ ions of the acid with the OH' ions of the base. On the other hand, the fact has been brought as an objection to the view. A weak acid is said to be such because it contains a less number of $H^{\cdot}$ ions than a strong one; hence, it is said, if the heat of neutralisation is due to the combination of these ions, it should be less in the former case. The nature of electrolytic dissociation as an equilibrium is lost sight of in this objection; as soon as the free ions, say of half the acid present, are neutralised, the remaining undissociated acid at once becomes half dissociated, its ions are then neutralised, and so on, until the whole of the acid has passed through the stage of ions and all the hydrogen ions have combined with the hydroxyl ions of the base.

To return to the question of *strong and weak acids*. We remember that the reason why hydrochloric acid is so much stronger than acetic acid in the same concentration is because the former is so much more highly dissociated. Since in very great degrees of dilution even weak acids are almost completely dissociated, it is clear that the difference between strong and weak acid becomes less as the concentration is diminished. While, therefore, it is sufficient, in order to define the acidity of a particular solution, to state the value of its concentration in hydrogen ions, it is useful to be able to compare the strength of different acids by numbers independent of concentration.

This can be done, in the case of a large number of acids, by means of their *dissociation constants*. To understand the significance of these values, we must, at some risk of repetition, refer to the law of *Mass action*. The historical development of this law will be dealt with in Chapter X., and a brief description only will be given here. The law in its simplest form states that the rate at which any reaction proceeds is directly proportional to the amount, or rather concentration, of the reacting substances. We have already seen cases where the whole mass of a substance present is not concerned in the chemical reaction, as, *e.g.*, in heterogeneous systems, where the "active mass" depends on the surface, but, if we understand "mass" in the above statement of the law to mean the mass actually taking part in the reaction, we may regard it as unconditionally true for all kinds of reactions. It is, of course, unnecessary to remark that the actual rate of any particular reaction depends on all kinds of conditions, which can be grouped together in the form of a constant (K), as long as they remain unchanged.

The law of mass action means that, other things remaining constant, doubling the concentration of any one of the reacting substances doubles the rate of the reaction, so that, if two are doubled, the rate is four times as fast, and so on. The necessity of this fact on the kinetic theory is obvious. Thus, the rate at which a reaction goes on depends on the number of collisions, per unit of time, that occur between the reacting molecules. Clearly, if the number of one kind of these molecules in a given space is doubled, the number of collisions is doubled, and if, also, the number of the other kind is then doubled, this rate itself will be doubled; so that the effect of doubling the concentration of both is to multiply by the rate due to the increase of both, that is, by four.

It is usual to express the concentrations of the reacting substances by the use of brackets: thus the rate of the reaction:—

$$A + B \rightleftarrows C + D$$

in which A and B react with the production of C and D, while C and D react to form A and B, is expressed as:—

$$K(A).(B) \rightleftarrows K'(C).(D) \quad \text{or}$$

$$K(C)_A.(C)_B \rightleftarrows K'(C)_C.(C)_D$$

A, B, C, D may stand for the concentrations of acetic acid, ethyl alcohol, ethyl acetate, and water, and the formula would then read:—

$$K(H\bar{A}).(\bar{E}tOH) \rightleftarrows K'(\bar{E}t\bar{A}).(H_2O) \quad \text{or}$$

$$K(C_{acid}) \;.\; (C_{alcohol}) \rightleftarrows K'(C_{ester}) \;.\; (C_{water}),$$

where K and K' are the velocity constants of the two reactions respectively. We note further that the ratio of these two quantities will define the composition of the system in equilibrium; if one reaction proceeds twice as fast as the other, it will be clear that, in order to bring up the rate of the slower reaction to that of the faster, as must be the case in equilibrium, the concentration of the reacting substances in its case must be correspondingly increased.

Now it was pointed out by Arrhenius that electrolytic dissociation must be governed by the law of mass action. In order to understand its application to this case, let us consider the ethyl acetate reaction in equilibrium, thus:—

$$(\text{alcohol})\ (\text{acid}) = K\ (\text{ester})\ (\text{water}),$$

where K is the ratio of the two velocity constants of our previous formulæ and is known as the "*equilibrium constant,*" and the names in brackets mean the respective concentrations of these substances. Suppose that we increase the concentration of any one of the components, it is easy to see that it involves simultaneous changes in all the others; for example, if we increase water, ester is diminished, in order to maintain constant value of the product, and ester cannot be decreased without increase of acid and alcohol. Perhaps the matter will be made clearer if we put the equation given above into the form:—

$$K = \frac{(\text{alcohol})\ (\text{acid})}{(\text{ester})\ (\text{water})}.$$

If water is increased, the value of the fraction may be kept constant by increase of either alcohol or acid, but neither of these can occur without the other nor apart from hydrolysis of part of the ester.

Take next acetic acid in water; the reversible reaction is:—

$$H\bar{A} \rightleftarrows H^{\cdot} + \bar{A}'$$

and, by mass action:—

$$K(H\bar{A}) = (H^{\cdot}).(\bar{A}') \text{ or } K = \frac{(H^{\cdot}).(\bar{A}')}{(H\bar{A})},$$

K being the equilibrium constant.

Put a = degree of dissociation, so that if $a = 0{\cdot}5$, half the molecules of the

acid are dissociated; then, if V is the volume of the solution containing one molecule of the electrolyte:—

$(H^{\cdot}) = \frac{a}{V}$, and also $(\bar{A}') = \frac{a}{V}$, since they are equal to each other, and $(H\bar{A}) = \frac{1-a}{V}$.

Therefore:—

$$K = \frac{a}{V} \times \frac{a}{V} \div \frac{1-a}{V} = \frac{a^2}{V(1-a)}.$$

This result was worked out by Ostwald (1888), and is known as his "*Dilution Law.*" It is found experimentally to apply to weak acids and bases. To salts, strong acids, and bases a different law applies, a law which is not dependent on mass action, as described above (page 182).

It will be seen that, when the dilution law applies, the constant K (known as the "*dissociation constant*" or "*affinity constant*") is independent of dilution and is valuable in comparing the strength of the electrolytes concerned. The following series may be found useful. The basic constants, of course, indicate the strength as bases, and are obtained from the concentration in OH′ ions. The substances with both acidic and basic properties are known as "amphoteric," and will be discussed later.

TABLE OF DISSOCIATION CONSTANTS

Substance.	Acidic Constant.	Basic Constant.	Authority.
Trichloracetic acid	121×10^{-2}	...	Ostwald (1889, p. 178)
Oxalic acid	10×10^{-2}	...	,, p. 281
Dichloracetic acid	$51{\cdot}4 \times 10^{-3}$	...	,, p. 177
Maleic acid	$11{\cdot}7 \times 10^{-3}$	...	,, p. 380
Trichlorlactic acid	$46{\cdot}5 \times 10^{-4}$	...	,, p. 194
Monochloracetic acid	$15{\cdot}5 \times 10^{-4}$	...	,, p. 176
Salicylic acid	$10{\cdot}2 \times 10^{-4}$	...	,, p. 247
Tartaric acid	97×10^{-5}	...	,, p. 372
Mandelic acid	$41{\cdot}7 \times 10^{-5}$	...	,, p. 272
Lactic acid	$13{\cdot}8 \times 10^{-5}$	...	,, p. 191
Aspartic acid	$6{\cdot}9 \times 10^{-5}$	$1{\cdot}3 \times 10^{-12}$	Winkelblech (1901, p. 587)
Succinic acid	$6{\cdot}6 \times 10^{-5}$	...	Ostwald (1889, p. 282)
Benzoic acid	$6{\cdot}0 \times 10^{-5}$	...	,, p. 246
Acetic acid	$1{\cdot}8 \times 10^{-5}$	...	,, p. 174
Caproic acid	$1{\cdot}45 \times 10^{-5}$	...	,, p. 176
m-Amidobenzoic acid	$9{\cdot}6 \times 10^{-6}$	$1{\cdot}9 \times 10^{-11}$	Winkelblech (1901, p. 587)
Carbonic acid (1st constant)	$3{\cdot}2 \times 10^{-7}$	...	Ostwald (1897, p. 159)
Arsenious acid	$6{\cdot}3 \times 10^{-10}$	$1{\cdot}0 \times 10^{-14}$	Wood (1908, p. 411)
Leucine	$3{\cdot}1 \times 10^{-10}$	$2{\cdot}7 \times 10^{-12}$	Winkelblech (1901, p. 587)
Phenol	$1{\cdot}3 \times 10^{-10}$	...	Lundén (1908, p. 83)
Glucose	$5{\cdot}1 \times 10^{-13}$	...	,, p. 83
Urea	...	$1{\cdot}5 \times 10^{-14}$	,, p. 85
Amino-azo-benzene	...	$8{\cdot}9 \times 10^{-12}$	,, p. 86
Aniline	...	$1{\cdot}1 \times 10^{-8}$	Winkelblech (1901, p. 586)
Glyoxaline	...	$1{\cdot}2 \times 10^{-7}$	Lundén (1908, p. 87)
Ammonium hydroxide	...	$2{\cdot}3 \times 10^{-5}$	Winkelblech (1901, p. 586)

Physiological Action of Hydrogen and Hydroxyl Ions.—The great activity of these ions in physiological processes will be seen in various phenomena to be described in later pages. This activity is undoubtedly in many cases connected with their great rate of migration, as compared with other ions. It has been suggested that this unusual rate is due to a special effect on the molecules of the solvent.

We have already had occasion to refer to the action of even very small concentrations of H· or OH′ ions on the sign of the electrical charge of colloidal particles. Especial attention may also be called to the great sensitiveness of enzymes in this respect, probably in great part due to the colloidal nature of these

agents. Means will be indicated later by which changes in acidity due to the products of their activity may be neutralised, and their activity kept constant, in so far as it is affected by this change of acidity.

Even enzymes such as emulsin, which do not, like pepsin or trypsin, require fairly strong acid or alkaline reaction, are greatly affected in their rate of action by changes such as are brought about by the addition of blood serum. This is not generally recognised in testing for the presence of "anti-enzymes," and has led to the belief in their existence when the result obtained was due merely to reduction of H· ion concentration (see Bayliss, 1912, 2, pp. 460-462).

The heart of the frog is affected by so small a change of H· ion concentration as that from neutrality ($10^{-7\cdot7}$) to one of $10^{-6\cdot5}$, and is killed by one of 10^{-6} (Fig. 55). On the alkaline side, an H· ion concentration of 10^{-10} is fatal. The addition of 0·036 mgm. of hydrochloric acid to 1 litre of distilled water would raise its H· ion concentration from $10^{-7\cdot7}$ to 10^{-6}.

The respiratory centre is extremely sensitive to very minute changes in the carbonic acid pressure of the blood, *i.e.*, in all probability, to changes in H· ion concentration from dissociation of H_2CO_3.

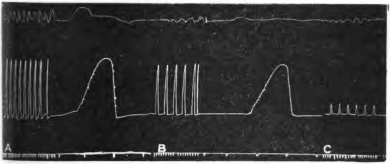

FIG. 55. EFFECT OF ACID ON THE ISOLATED HEART OF THE FROG.

A. Perfused with normal Ringer's solution, with H· ion concentration of $10^{-7\cdot7}$.
B. After perfusion for twenty minutes with faintly acid Ringer's solution, H· ion concentration, $10^{-6\cdot5}$.
C. After perfusion of the acid solution for eighty minutes. The upper curve in each is that of the auricle. The lower one, that of the ventricle. The signal gives time in seconds. As shown by the time signal, the rate of movement of the surface was quickened on two occasions in order to show details of the curves better.

(Clark, 1913, 1.)

These facts will suffice to show the importance of two things to which we must devote some attention. The first is the means of exact measurement of the H· ion concentration of a solution, the second is the capacity possessed by the blood and the cells to neutralise even considerable addition of acids or alkalies, in order to maintain the state of nearly complete neutrality which is essential.

If we were dealing with distilled water only, the addition of one-millionth of a gram-molecule of hydrochloric acid to a litre would raise its H· ion concentration from $10^{-7\cdot7}$ to 10^{-6}, that is more than ten times, a change that would be fatal to many delicate protoplasmic processes. The mechanism which prevents such a result will be described later.

Measurement of Hydrogen Ion Concentration.—Very brief consideration will suffice to show that, in the case of weak acids and bases, or even strong ones in concentrated solutions, the ordinary methods of *titration* by adding a standard solution of acid or base until a certain change in a coloured indicator is produced, although giving valuable information as to the total concentration of free acid or base (*i.e.*, dissociated plus undissociated), are not sufficient to afford the desired data as regards the H· ions of the dissociated fraction. Different acids, as we have seen, vary considerably in their degree of dissociation in equimolar solutions. This dissociated part is

always a certain proportion of the total acid present, so that the moment a part of the acid has been removed by the addition of a base, the remaining acid undergoes a further dissociation and so on, until the whole of the acid, whatever its original dissociation was, has become completely dissociated and its hydrogen ions have entered into combination with the hydroxyl ions of the base.

There are, however, certain methods by which the actual hydrogen ion concentration can be estimated without causing any change in it.

We will first consider the use of *Indicators*. These are certain dyes which have a particular colour at a certain concentration in $H^{\cdot}$ ions and another colour at another concentration which differs very little from the first. Those which change colour at points not far distant from neutrality are the most useful, especially in physiological work.

That it is really the hydrogen ion concentration that these substances "indicate" is obvious if we take a series of five dilutions of hydrochloric acid, viz., twice normal, normal, deci-, centi-, and milli-normal; the colour of crystal violet will be found to be yellow in the first, yellow-green in the second, blue-green in the third, blue in the fourth, and violet in the fifth. No alkali has been added, and the only difference between the various solutions is the concentration in the acid.

The whole question of the theory of indicators cannot be entered into here, but may be found in Prideaux' book (1920). Generally speaking, they are salts of either a very weak acid or a very weak base, sometimes the free acid or base itself. The change in colour is due to the electrolytic dissociation of the salt with the production of an ion which has a different colour from that of the free undissociated acid or base.

Since the strength of the indicator acid or base varies in the different substances used for the purpose, it will be clear that the acidity of a given solution may be determined by the use of a series of indicators changing colour at different $H^{\cdot}$ ion concentrations. In theory, the question is a little complicated by the existence of what are known as "pseudo-acids," which have a different chemical structure in the free state to that in their electrolytically dissociated salts; but the explanation given, which was originally due to Ostwald, is not practically altered by this fact.

That indicators do actually vary in the acidity of the solution to which they respond can easily be seen by comparing methyl orange with phenolphthalein. If a solution of hydrochloric acid be taken it will be found that methyl orange is red in it. Alkali is now added until the colour changes to orange, that is, the solution is alkaline to this indicator. If another sample of the acid be taken, it will be found to produce no colour with phenolphthalein, and *more alkali* must be added to change the colour of this indicator to the red one of its salts than was required to change the colour of methyl orange.

In the use of indicators there are several precautions to be observed.

In the first place, the hydrogen ion concentration at which certain of them change colour is not the same in pure acids or bases as in the presence of foreign substances, especially salts and proteins. For a description of these cases, the reader is referred to the investigations of Sörensen (1909), which are concerned with the various methods of practical use for the estimation of hydrogen ion concentrations. The use of indicators for physiological purposes will be found fully treated. In the second place, it will be obvious that the total amount of the indicator present must not be so great as to neutralise, or react with, any perceptible portion of the ions to be estimated.

This will be made clear if we take a dilute solution of Congo-red, the sodium salt of an acid whose coloured ion is red and whose undissociated free acid is blue. Add a drop of this solution to a very dilute solution of hydrochloric acid, a blue colour is given. Take again a concentrated solution of the indicator and add it in rather large amount to a small quantity of the very dilute acid. The colour will remain red, because the whole of the free hydrochloric acid present has been used up to combine with a portion only of the dye, and the colour of the salt still left in excess masks the bluish colour of the very small amount of the free dye-acid. This fact is especially liable to mislead when test papers are used, and a drop of very dilute solution, or one containing only a very small amount of hydrogen ions, is applied to the paper, as has been pointed out by Walpole (1913, 1). In such cases the reaction will appear to be different when a drop is placed on the paper and when the paper is immersed in a large volume of the solution.

Walpole (1910) has also described an ingenious artifice by which it is possible to use an indicator with solutions containing coloured substances. This method consists essentially in comparing the colour of the solution to which an indicator has been added with that of the light which has first passed through an equal depth of the coloured solution alone, and

afterwards through water containing the indicator alone. When used for titration, for which purpose the arrangement is particularly adapted, the acid or alkali is added to the cell containing the indicator alone until the change in colour corresponding to the required concentration in H· ion is obtained. This cell is then observed by light which

Indicator	Colour of indicator with Hydrogen ion Concentration of N × 2	1	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}	10^{-11}	10^{-12}	Concentration of Indicator Solution	Drops added to 10 c.c. of test
Methyl-violet. 6B. = Crystal-violet	gold yellow	green	green blue	blue	violet										0·05%	3-8
Tropoeolin 00 = Diphenylamine-orange			red	flesh	yellow										0·01%	3-5
Benzyl-anilino-azo-benzene. Sörensen. p. 99				red	orange										0·01% in 90% alcohol	5-10
(Dimethyl-amino-azo-benzene)				red	flesh	gold yellow									0·01% in 80% alcohol	5-10
Methyl-orange = Tropoeolin D.				red	orange red	orange	yellow								0·01%	3-5
(Congo Red)					blue	violet	scarlet								0·01%	3-5
Methyl-Red					violet red	red	orange	yellow							0·02% in 60% alcohol	4
Paranitrophenol							none	faint green	green yellow						0·04% in 6% alcohol	3-20
Neutral Red	blue	blue violet	red						rose	orange	yellow				0·01% in 50% alcohol	10-20
α-Naphthol-phthalein									none	greenish	blue				0·04% in 60% alcohol	4-12
Tropoeolin 000. I. = α-naphthol-orange									yellow	orange red	red				0·01%	4-10
Phenol-phthalein										none	rose	red			0·05% in 50% alcohol	3-20
Thymol-phthalein												none	blue		0·04% in 50% alcohol	3-20
Tropoeolin O = Resorcinol-yellow													green yellow	orange	0·01%	5-10

FIG. 56. TABLE OF INDICATORS, COMPILED FROM THOSE OF SALM (1906) AND OF SÖRENSEN (1909).—The double vertical line implies that the change of colour is sharpest at these points. The gaps are to be understood as filled with the name of the colour next adjoining; for example, phenolphthalein is colourless in all concentrations of hydrogen ions greater than 10^{-9}, red in all those lower than this.

has passed through a depth of the coloured solution equal to that to which the indicator has been added. Acid or alkali is then added to the latter until its colour is the same as that of the combination of the coloured solution with the indicator solution in separate vessels. The absorption due to the coloured substance is obviously identical in the two cases.

The table given in Fig. 56, which is extracted from the results of Salm (1906) and of Sörensen (1909), may be useful. With the exception of those indicators

in brackets, it contains only those found by the latter investigator to be unaffected by the presence of moderate amounts of such substances, proteins or neutral salts, as are likely to be present in physiological solutions. I have omitted two of those recommended by Sörensen on account of the difficulty of obtaining them, and have inserted in place of them, where the series would otherwise be incomplete, other indicators in common use, but more sensitive to the disturbing presence of neutral salts and proteins. These are marked by brackets.

Neutral red is an extremely valuable indicator for many physiological purposes. It changes colour at the neutrality of water, and has obvious changes at points just above and just below this concentration in hydrogen ions. It is practically unaffected by the presence of protein and is innocuous to living protoplasm.

The *cautions* to be exercised when neutral salts or proteins are present in any considerable quantity may be found in the paper by Sörensen (1909, pp. 72-120). Attention may be called to phenol- and thymol-phthaleins as being least affected thereby, and especially to the new indicator, a-naphthol-phthalein, which changes colour between the H· ion concentrations of $10^{-7\cdot 26}$ and $10^{-8\cdot 66}$, *i.e.*, a very little on the alkaline side of neutrality (Sörensen and Palitzsch, 1910).

The Hydrogen Electrode.—This method, although somewhat elaborate in the apparatus required, and demanding careful work if small differences in H· ion concentration are to be measured, is the most direct and the least liable to disturbance by foreign substances.

In order to understand the principle of it, the reader may be glad of a few words on the *theory of electrode potential.*

When a solid is placed in water, it has a certain tendency to send off its molecules into the water so as to form a solution. The intensity of this varies greatly in different cases, and is known as the solution pressure of the substance in question. It occurred to Nernst (1889, pp. 150-151) that the electrical phenomena shown by metals immersed in solutions of their own salts might be treated quantitatively from a similar point of view, on the assumption of the truth of the electrolytic dissociation theory. When a metal, say copper, is immersed in a solution of one of its own salts, say the sulphate, the copper has a tendency to give off Cu·· ions into the solution. There are already ions of the same kind in the solution, which, by their osmotic pressure, oppose the passage of similar ions from the metal. The force with which the metal tends to send out ions into the solution is called by Nernst its "*electrolytic solution pressure,*" and may be greater or less than the osmotic pressure of the metallic ions in the solution. It will be plain that, in the former case, the metal will become negatively charged, owing to its giving off positive charges on the ions which leave it. Its potential will depend on the difference between its electrolytic solution pressure and the osmotic pressure of the ions in the solution. If the latter is the greater, the electrode will have a positive charge, owing to the receipt of positive ions from the solution. It is to be remembered that the ions given off from the metal cannot travel beyond an infinitesimal distance from the oppositely charged mass of metal, owing to electrostatic attraction, as has been pointed out above.

It is obvious that we cannot make use of any one of these electrodes alone, since we must have metal at both ends of our cell in order to form the circuit for the purpose of measurement. If we form our battery by joining up two electrodes of the same metal in solutions of the same concentration, there will be no electromotive force in the combination, since the two electrode potentials are equal and in opposite direction to one another. If, however, the concentrations of the metallic ion in the two solutions are unequal, the electromotive force of the battery is equal to the difference between that of the two electrodes. This arrangement is known as a "*concentration battery.*" If we know the concentration of one of the solutions, and can measure the electromotive force of the combination, we can obtain the concentration of the other solution by difference, supposing that we know the law which governs the relation between the potential and the concentration of the solution. Now it has been shown by Nernst (1889), originally from thermodynamic considerations, although the assimilation by van't Hoff of solutions to the gas laws would lead to the same result, that this relation is given by a similar expression to that for the work

done in compressing a gas isothermally from a pressure p to P. This is, as we have seen (page 35 above),

$$RT \log_e \frac{P}{p}.$$

We may, in fact, regard the two pressures of the formula as being the osmotic pressure of the metallic ions of the solution (p) and the electrolytic solution pressure of the metallic electrode (P). We have, then, merely to express the terms of this formula in the appropriate electrical units in order to obtain the relation between potential and concentration of ions in the solution. This is done by dividing by the charge in coulombs on one gram ion, the Faraday constant; by doing this, we convert pressure in mechanical units into electrical force. If the ion in question is multivalent, the Faraday constant (F) must naturally be multiplied by the number of charges carried, that is by the valency (n). R, the gas constant, must also be expressed in electrical units. We have, then:—

$$\frac{RT}{nF} \log_e \frac{P}{p}.$$

R, in electrical units, is 8·3, and F, in coulombs, is 96,540, so that, at the temperature of 18° ($=273+18$ absolute), the value of $\frac{RT}{F}$, when multiplied by 2·3 to allow the use of ordinary logarithms, becomes—

$$\frac{8\cdot3 \times 291 \times 2\cdot3}{96{,}540} = 0\cdot058.$$

Another method of calculating this number will be found in the book by Nernst (1911, p. 753).

We need, then, only to know P, the electrolytic solution pressure of the metal used, in order to be able to determine p, the osmotic pressure of the ions in the solution and, therefore, their concentration. P has been determined for a number of metals. In the case of a concentration battery, it is eliminated thus:—

The total electromotive force of the combination is

$$\frac{RT}{nF}\log_e\frac{P}{p_1} - \frac{RT}{nF}\log_e\frac{P}{p_2} = \frac{RT}{nF}\log_e\left(\frac{P}{p_1} \div \frac{P}{p_2}\right) = \frac{RT}{nF}\log_e\frac{p_2}{p_1} \text{ or } -\frac{RT}{nF}\log_e\frac{p_1}{p_2},$$

where p_1 and p_2 are the respective concentrations of the two solutions.

We may note that the electrolytic solution pressure may be looked upon as that osmotic pressure of the ions in the solution which just balances the tendency of the ions of the electrode to pass out; so that the electrode would have zero potential if it were possible to obtain a solution of the correct concentration.

Certain metals, such as platinum and copper, have a very low electrolytic solution pressure, so that they are always positive in solutions of their salts, and it will be clear that the higher the concentration of the salt is, the greater will be its tendency to send positive ions into the metal, or, in other words, the greater will be its potential. Zinc, on the other hand, is an example of a metal with a very high electrolytic solution pressure, so that the osmotic pressure of the ions in solutions of its salts will always be lower than its own; in this case the potential will be higher, the lower the concentration of the solution, since it is due to the sending out of ions by the electrode.

We may now proceed to the description of the *hydrogen electrode*. It will have been sufficiently obvious from the preceding pages that, if we could make an electrode of this gas and immerse it in a solution containing hydrogen ions, that is, an acid solution, we should have the means of measuring the concentration of the hydrogen ions by the potential of the electrode. It will probably occur to the reader that, if we saturate palladium with hydrogen, we have what is required so long as our solution does not attack the metal chemically. It will, of course, be remembered that the potential is determined only by ions common to both electrode and solution. Palladium, however, is attacked by some acids which we require to take account of—hydrochloric acid, for example. We must therefore use platinum, which also takes up hydrogen, although in less amount than palladium does, so that it needs more care to saturate it and keep it saturated. In practice, the electrode is sometimes made of gold, merely plated with platinum

black, in order that it may be rapidly saturated with hydrogen. The gold, of course, merely serves as a conducting support for the platinum.

It is unnecessary for both electrodes to be hydrogen electrodes, or to have a concentration battery in hydrogen, although in some cases it may be desirable. So long as the opposing electrode is of a known electromotive force, it may be of any form. In practice, the Ostwald calomel electrode, described on p. 202 of Findlay's book (1906), is generally used. The tables given in the paper by Schmidt (1909) will be found to save much time in calculation.

There is one circumstance to be taken into consideration which has so far been omitted, for simplicity, in our account. We saw above (page 178) that when the two ions of an electrolyte have different velocities, there is a difference of potential at the contact surface of such a solution with water, and also when two solutions of different concentrations are in contact. This electromotive force is allowed for in the complete Nernst formula for a concentration battery by the factor—

$$\frac{u-v}{u+v}\mathrm{RT}\log_e\frac{c_1}{c_2},$$

where u and v are the mobilities of the two ions in question, and c_1 and c_2 the concentrations of the two solutions in contact; R and T have their usual meaning (Nernst, 1911, p. 752).

In the case of the complex physiological solutions with which we often have to deal, calculations on the basis of this expression are practically impossible, since we are uncertain as to the actual ions concerned. The contact difference is therefore rendered as small as possible by the interposition of a saturated solution of potassium chloride in the manner described by Bjerrum (1905), between the solutions of the two electrodes. It appears that the great excess of ions, having very nearly the same rate of migration, makes the two contact potential differences between this solution and the solutions in the electrode vessels practically equal and opposite to one another, while the dissociation of the electrode solutions is greatly diminished at the contact. When great accuracy is required, determinations are made of the total electromotive force of the combination when potassium chloride solutions of different concentrations are interposed. From the data obtained the true value can be determined by extrapolation. Other very soluble salts, such as ammonium nitrate, are sometimes used.

The *measurement* is made by a compensation, or potentiometer, method. A wire, best made of platinum-iridium, is stretched along a scale, and through it a current is passed from a constant battery, such as a partially discharged storage cell. By means of a sliding contact, any fraction of the electromotive force between the two ends of this wire can be tapped off and opposed to that of the electrodes until the whole is brought to zero. Some means of detecting this point of balance is necessary, and, owing to the high resistance usually present in the circuit, the capillary electrometer, to be described in Chapter XX., is generally used. The value of the reading on the scale of the slide wire is obtained by determining at what reading the electromotive force of a standard cell is balanced. The value of each scale division is then known.

For further practical details the reader is referred to Findlay's book (1906), for the general method, and to the paper by Sörensen (1909) for the physiological applications. A diagram of the circuit is given in Fig. 204 (Chapter XXII.).

The most important of these applications may now be referred to, that of estimating the true *hydrogen ion concentration of the blood*, which it is impossible to determine in any other way. The difficulty here is that a part of the hydrogen ions arise from carbon dioxide dissolved in the liquid, so that, if the usual method of passing hydrogen gas through the solution in which the platinum electrode is immersed for a part of its area be used, carbon dioxide gas is driven off and the acidity decreased. In the earlier determinations of the reaction of the blood this circumstance was not duly taken into account. The difficulty is obviated by taking a closed volume of hydrogen in contact with the electrode, which has been previously saturated with it, shaking this limited volume of gas with a portion of blood, so that the carbon dioxide tension of the gas phase becomes equal to that of the liquid. This blood, which has lost a part of its carbon dioxide, is replaced by a fresh portion, which will need to part with only a minute fraction of its carbon dioxide to the hydrogen. This was first done by Michaelis, and an improved method has been described by Hasselbalch (1910). More recently, Walpole

(1913, 2) has invented a simple form of hydrogen electrode, which can be used for

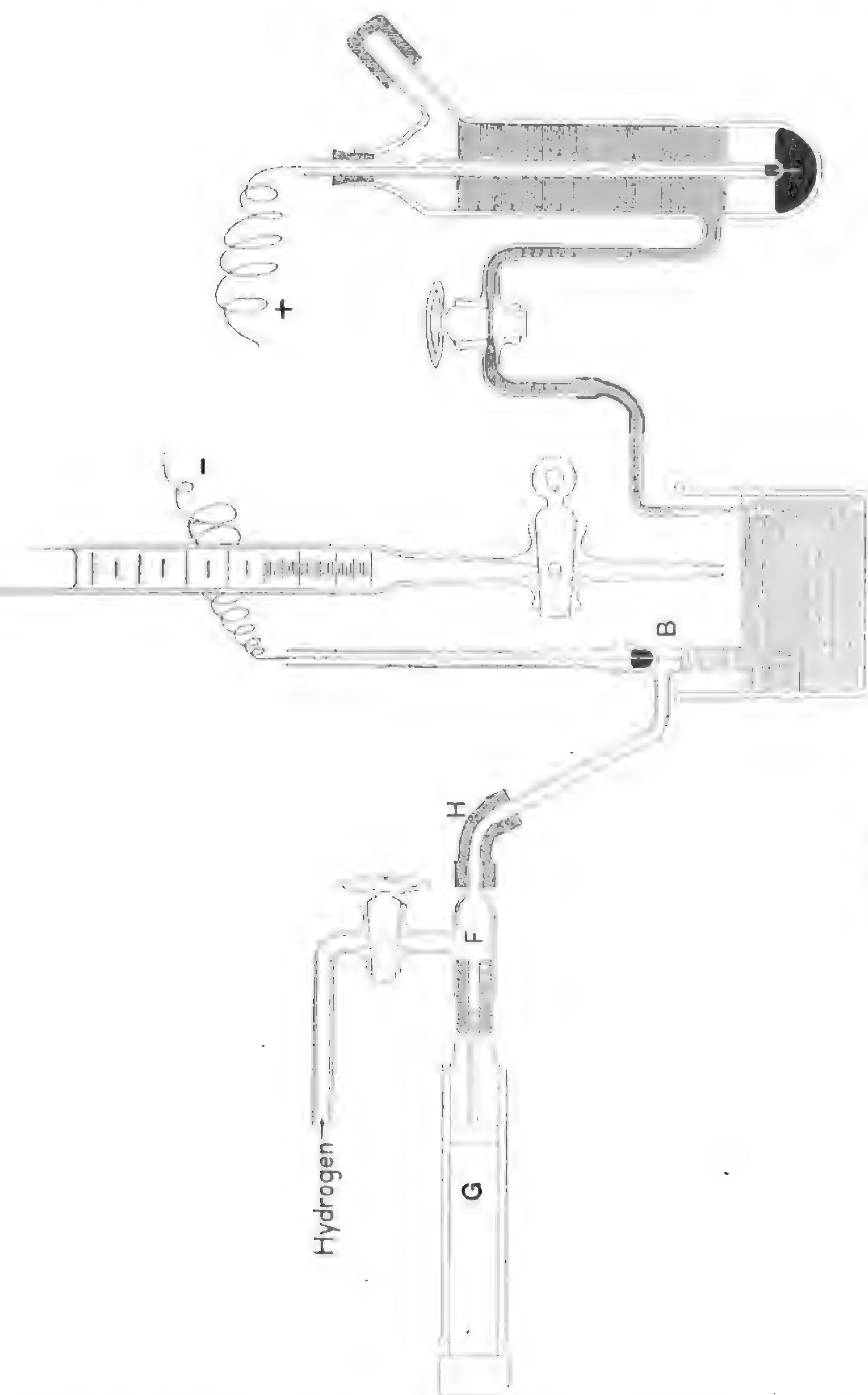

FIG. 57. WALPOLE'S HYDROGEN ELECTRODE.

G, Glass syringe to suck up solution into *B*, Hydrogen electrode proper, of about 3 c.c. capacity. Fine platinum wire just touching the surface of solution. On the right is the Ostwald calomel electrode. The burette is for the purpose of titrating an acid or alkaline solution electrometrically.

(Walpole, 1913, 2.)

various purposes; with care, it can be made to serve the purpose of the Hasselbalch form. Fig. 57 shows the Walpole electrode

In a later paper Walpole (1914, 1) describes improvements in this electrode. Peters (1914) uses another excellent form. But the best is that of M'Clendon and Magoon (1916), or that of W. M. Clark (1915).

In the case of blood, or other solution containing hæmoglobin, there is another difficulty. Platinum takes up oxygen as well as hydrogen, and, in pure oxygen, it serves as a hydroxyl ion electrode, although not so accurately defined as the hydrogen one, owing to its sensibility to various disturbing conditions. When in use as a hydrogen electrode, it is obvious that the potential which it assumes in a solution of given hydrogen ion concentration will not be the same if the gas in contact with it contains oxygen, as must be the case if shaken with a solution of oxyhæmoglobin. At present it seems impossible to devise a method of removing oxygen without producing other changes in the blood. Perhaps carbon monoxide would serve.

The *general method* of determining the concentration of particular ions in a solution by the use of appropriate electrodes is probably capable of wider application in physiology than it has yet received. Thus the changes in the concentration of chlorine ions due to separation and dissociation of chlorides and changes in the tension of oxygen can be investigated on these lines. These are processes which occur in physiological activity, and Roaf (1913) has already obtained valuable information with regard to changes in contracting muscle by these methods. Reference will be made to these results later.

In the description of the Nernst theory of the metallic electrode, it must not be forgotten that the process is not the same as that of ordinary solution. Owing to the forces of electrostatic attraction, the ions given off from the metal cannot actually pass beyond the immediate proximity of the electrode itself, thus giving rise to a Helmholtz double layer. The case of a solution enclosed by a membrane permeable only to one of the ions into which the solute dissociates is a completely analogous one. The surface of the metal itself in the Nernst electrode may be regarded as permeable to its own positively charged ions, but not to the oppositely charged mass of metal. The former ions, however, are held fast by electrostatic attraction until the circuit of the battery is completed, when they are able to pass out from the one electrode, which is dissolved, and are deposited on the opposite one, losing their charges and increasing the mass of the metal.

There is one point in connection with the Nernst formula which may have struck the reader, although it is not alluded to in the usual descriptions of the theory. It had, however, not escaped the notice of the original author (Nernst, 1911, p. 139). If p_1 in the expression:—

$$\frac{RT}{F}\log_e\frac{p_2}{p_1},$$

becomes zero, *i.e.*, if the liquid in one electrode is in infinite dilution, or, in other words, is water, the value of the potential difference becomes infinite. Nernst points out that, theoretically, the diffusion of any substance into a space which is, for it, a vacuum, should take place with infinite velocity. In the case of a gas this condition would last only for an infinitesimally short time. Water is practically never a vacuum for electrolytic diffusion, since there are always ions in it. There are, moreover, other reasons connected with the conditions at the surface, which make measurements with solutions of less than 0·001 molar strength unreliable as indicating the state of the solution as a whole (see the remarks by Nernst referred to above).

Certain *other methods of estimating the hydrogen ion concentration* of a solution require a brief account. These are of a more chemical nature, and are occasionally useful. As a rule they necessitate a previous knowledge of the composition of the solution apart from its concentration in hydrogen ions.

Hydrolysis of Esters.—The rate at which methyl or ethyl acetic esters are hydrolysed in water is found to be proportional to the hydrogen ion concentration present. It may be used as a convenient method for the comparison of fairly high concentrations of these ions, but with weak acids the rate is too slow to be of much practical value. The presence of *neutral salts* affects the rate of the reaction in an anomalous way. If we return for a moment to the equation for the dissociation of a weak acid in equilibrium with its ions, viz.:—

$$K(C)_{H\bar{A}} \rightleftarrows (C)_{\bar{A}'} \times (C)_{H^{\cdot}} \text{ or } K = \frac{(C)_{\bar{A}'}.(C)_{H^{\cdot}}}{(C)_{H\bar{A}}},$$

it will be seen that any increase in the concentration of the acetic ion leads to diminution in that of the hydrogen ion, in order that K may remain constant. This increase may be produced by the addition of a salt of the weak acid, in our case say sodium acetate, which dissociates into acetic and sodium ions. Experimentally this is found to be the case. It is, indeed, a deduction from the law of mass action. But it does not apply to strong acids and their salts. In fact, the addition of sodium chloride to a solution of hydrochloric acid *increases*,

instead of decreasing, the hydrolysis of an ester by the solution. This difficulty in the electrolytic dissociation theory was noticed by Arrhenius himself (1889, 2, and 1899), and called "*neutral salt action.*" It shows that neutral salts of a strong acid increase the effect of the acid itself in some way not yet clear. Attention has already been called to the anomalous behaviour of salts, strong acids, and strong bases, and the views of Noyes, etc., on the question (see page 182 above). Some suggestions made by Senter (1910), at the conclusion of a paper which bears on the subject, may be of interest. The influence of neutral salts may be supposed to be exerted on the water or on the substance being hydrolysed, sugar or ester. In the former case the dissociation may be increased, or the action may be of some unknown kind on the non-dissociated molecules. In the latter case the effect may be due indirectly to an effect on the dissociative force of the medium. Senter himself favours the latter view, but regards it as probable that there may be several causes acting together. Possibly hydration of the ions of sodium chloride may increase the effective concentration of both acid and sugar, but it is doubtful whether the effect would be large enough.

Of the various hypotheses made in explanation of this effect, those of Caldwell (1906), Snethlage (1913), and Taylor (1914) may be referred to. According to Caldwell, the action of salts in increasing the rate of hydrolysis by acids is to be accounted for by a real increase in concentration of the acid. This takes place in two ways. If volume normal solutions are taken, a part of the water is displaced by the molecules of the salt, in the sense of van der Waals' constant, b. These salts also actually take up water in some way, so that it is rendered unavailable for dilution of the acid; so that, again, the amount of water really free is less than it appears to be. Determinations of the increased quantity of water required to bring the rate of hydrolysis to the same value as that in the absence of salt leads to values of the amount used in "hydration" of the salt very close to those found in other ways, as will be described in the next chapter. Snethlage's work, in Bredig's laboratory, suggests that the undissociated part of the acid has also a catalytic action in the hydrolysis of esters and cane-sugar. As the affinity constant of the acid rises, so does the catalytic power of the undissociated part. In the weakest acids, that of the undissociated molecules is less than that of the hydrogen ions, but in the strong acids it may actually be greater. The action of chlorides in increasing the rate of hydrolysis of cane-sugar by hydrochloric acid is thus explained by the decrease of dissociation of the acid, as demanded by mass action on the Arrhenius theory. Taylor (1914) comes to conclusions similar to the last, in more detail. He also finds that the catalytic action of the undissociated acid increases with the affinity constant of the acid. If C_1 is the concentration of the hydrogen ion, C_2 that of the undissociated acid, k_H the catalytic action of the former, k_m that of the latter, then

$$\frac{k_m}{k_H} = \frac{C_1}{\sqrt{C_2}}.$$

It will be noted that it is not definitely known whether the H˙ ion concentration is actually raised by neutral salts.

As regards the part played by this "neutral salt action" in physiological phenomena, see the paper by Höber (1910, 3).

For very weak acids a sensitive method has been described by Fraenkel (1907). *Diazo-acetic-ester* is decomposed, with evolution of nitrogen gas, by very low concentrations of hydrogen ions, and is of use even in the case of the very weak amino-acids.

A method similar to that of hydrolysis of ordinary esters, and, like it, specially useful for the stronger acids, but subject to "neutral salt action," is the *inversion of cane-sugar*. This consists in the hydrolysis of the disaccharide, with the formation of glucose and fructose and, being associated with a considerable fall in the power of rotating polarised light, can be followed with the polarimeter in a convenient manner.

PRESERVATION OF NEUTRALITY IN THE ORGANISM.

We have seen how very sensitive the various processes, both chemical and physical, taking place in the organism are to changes in concentration of hydrogen ions. Now a large number of the reactions going on result in the production of such changes, and it is not to be supposed that it would be desirable that these changes should be entirely neutralised, even if it were possible. For example, the sensitiveness of the respiratory centre to slight increase of hydrogen ion con-

centration serves to get rid of the two products of muscular activity—carbon dioxide by the increase of respiratory ventilation, and lactic acid by increased supply of oxygen. At the same time, unless there were an efficient mechanism for moderating the changes in hydrogen ion concentration, there would be serious disturbance of the delicate action of protoplasmic processes.

This mechanism does in fact exist, and has been elucidated chiefly by the work of Lawrence J. Henderson, whose article on the subject (1909) should be consulted for a more detailed account than can be given here.

The possibilities of a means of soaking up, as it were, excess of hydrogen or hydroxyl ions would naturally be looked for in the more complex forms of electrolytic dissociation of the salts of the bi- or tri-valent acids, in combination with the hydrolytic dissociation of salts of weak acids with strong bases. This latter process has not been as yet discussed in these pages, and will require some consideration presently.

There are two systems of this kind to which early investigators turned their attention. They are both found widely spread throughout the animal organism. The first is that of the bicarbonates and carbon dioxide, which is to be met with chiefly in the blood, but also in the cells of the tissues generally. The second is that of the acid and alkaline phosphates, of greater importance in the cells. There are also, of course, interactions between the two systems, thus:—

$$Na_2HPO_4 + H_2CO_3 = NaH_2PO_4 + NaHCO_3,$$

so that there is always present a complex state of equilibrium between the two phosphates in addition to that between the bicarbonates and carbon dioxide. The proteins, as amphoteric electrolytes, and therefore capable of combination with both acids and bases, although, in all probability, only with strong acids and strong bases, except in rare instances, must also be taken into account. As we shall see, however, the part played by proteins appears to be comparatively unimportant. Adsorption, possibly, may also play a subordinate part.

In the further treatment of the question, I follow closely that of Lawrence J. Henderson.

We must remember that, contrary to what happens in simple homogeneous systems, such as true solutions in water, we have to deal in the blood and tissues with the complication due to phases and the phenomena, such as adsorption, which take place at their contact surfaces. It is well, however, to understand the less complex case to begin with. The results can afterwards be modified, if necessary, by the introduction of further factors.

It has long been known that the blood is able to withstand the addition of considerable amounts of free acid or alkali without much change in its reaction. This has been correctly described as being chiefly due to the carbonates and phosphates present, although the mechanism could not receive a satisfactory explanation until the electrolytic dissociation theory was propounded.

Let us consider first the *phosphate system.* The mono-sodium phosphate (NaH_2PO_4) behaves as a very weak acid owing to the way in which it dissociates, while the di-sodium phosphate (Na_2HPO_4) is a very weak base. The dissociation of these salts may be represented as taking place in stages, thus (marking the equations for convenience of future reference):—

$$(1)\quad Na_2HPO_4 \rightleftarrows Na^{\cdot} + NaHPO_4'.$$
$$(2)\quad NaH_2PO_4 \rightleftarrows Na^{\cdot} + H_2PO_4'.$$
$$(3)\quad NaHPO_4' \rightleftarrows Na^{\cdot} + HPO_4''.$$
$$(4)\quad H_2PO_4' \rightleftarrows H^{\cdot} + HPO_4''.$$
$$(5)\quad H_2O \rightleftarrows H^{\cdot} + OH'.$$
$$(6)\quad HPO_4'' + H_2O \rightleftarrows H_2PO_4' + OH'.$$

Hydrolytic Dissociation.—With respect to the two last equations, we note that the source of the OH' ions giving alkalinity to solutions of Na_2HPO_4 is the reaction in which the ion HPO_4'' combines with the $H^{\cdot}$ ion of water, leaving OH' in excess. The electrolytic dissociation of water itself has not yet been discussed,

but the evidence that such is the case is sufficiently strong to warrant us in making use of the phenomenon in the explanation of many facts, an explanation which it gives in a simple and reasonable way. The actual evidence itself will be given in the following chapter of this book.

A salt of a weak acid with a strong or weak base, or of a weak base with a strong or weak acid, that is, any salt of which one or both components is a weak one, is hydrolytically dissociated to a certain extent in water. There are present in the solution free acid and free base. In this connection the designation "strong" and "weak" should be understood in a somewhat relative sense. For example, ammonium hydroxide behaves as a weak base towards the strong acid, hydrochloric, but as a fairly strong base towards the very weak acid, leucine. I refer to this point here on account of the fact that salts of weak acids with weak bases are not so highly dissociated hydrolytically as might have been expected. The question will be discussed below.

In order to understand the process a little more detail is desirable. Remembering that the dissociation constant of an electrolyte expresses the proportion in which the non-dissociated part is capable of existing in the presence of its ions, let us see in the first place what happens when a strong acid, such as hydrochloric, is added to a solution of a salt of a weak acid, say to sodium acetate. Both of these are highly dissociated electrolytically, but when mixed, opportunity is given for the formation of two other electrolytes, sodium chloride and acetic acid, the former of which is highly dissociated, but the latter very feebly so. The low dissociation constant of acetic acid means that acetic ions and hydrogen ions can exist together only to a very small extent. Hence, in our mixture, they unite almost completely to form acetic acid, the result being that the hydrogen ions of the hydrochloric acid very nearly disappear. For practical purposes the reaction may be expressed thus:—

$$H^{\cdot} + Cl' + Na^{\cdot} + CH_3COO' = CH_3COOH + Cl' + Na^{\cdot}.$$

Further, owing to the great affinity of $H^{\cdot}$ for OH' ions, the minutest quantity only of either can exist in the presence of the other. Hence, the neutralisation of a strong acid by a strong base may be represented by an equation similar to that above:—

$$H^{\cdot} + Cl' + Na^{\cdot} + OH' = H_2O + Cl' + Na^{\cdot}.$$

Now water contains the small concentration of both $H^{\cdot}$ and OH' ions which can exist together. Applying the law of mass action to this equilibrium, $H_2O \rightleftarrows H^{\cdot} + OH'$, we have:—

$$K = \frac{C_{H^{\cdot}} C_{OH'}}{C_{H_2O}},$$

where $C_{H^{\cdot}}$, $C_{HO'}$, and C_{H_2O} are the concentrations of the $H^{\cdot}$ ions, the OH' ions and the water respectively. Since the latter is always very large in relation to the others, it may be taken as invariable, so that the *product* $C_{H^{\cdot}} \times C_{OH'}$ *is constant* in any aqueous solution. It is numerically equal to $1{\cdot}2 \times 10^{-14}$.

Water, then, is both a very weak acid and a very weak base; that is, it is what we shall learn later to call an "amphoteric electrolyte." When a neutral salt AB (using A' for the anion and $B^{\cdot}$ for the cation) is dissolved in water, there is the possibility of the formation of two new compounds with the ions of water, viz., HA and BOH. How far this will occur depends on the strength of the acid and the base. Suppose we take NaCl, the quantities of HCl and of NaOH will be very small, because of their great dissociation, and approximately equal quantities of $H^{\cdot}$ and OH' will be removed from the water for the purpose, being replaced by a slight further dissociation to keep $C_{H^{\cdot}} \times C_{OH'}$ equal to $1{\cdot}2 \times 10^{-14}$. Again, suppose that we take borax instead of sodium chloride. Here HA is a very weak acid, while BOH is a strong base. We have now in solution A', $B^{\cdot}$, $H^{\cdot}$, and OH' ions, and HA and BOH will be formed as before. But, since HA is very slightly dissociated, while BOH is highly dissociated, there will be excess of OH' ions. As before, a little water will dissociate, but only to preserve the equilibrium $C_{H^{\cdot}} \times C_{OH'}$ equal to $1{\cdot}2 \times 10^{-14}$, and this cannot get rid of the OH' ions, so that the solution will have an alkaline reaction. The case where the acid is strong and the base weak may be treated in a similar way, and the result will be

found to be that the solution has an acid reaction. Such a case is that of aniline hydrochloride. The degree of hydrolytic dissociation may be determined by methods involving the estimation of the concentration of hydrogen or hydroxyl ions, such as the hydrogen electrode, rate of hydrolysis of esters, etc.

The treatment of the subject given above is that of Philip (1910, p. 260). The book of Nernst (1911, pp. 530-533) may also be consulted with advantage. It will be noticed that the process essentially depends on the slight electrolytic dissociation of weak acids and weak bases.

From the equation for the reaction constant of hydrolysis given by Nernst (1911, p. 531), which is—

$$\frac{K_4}{K_2 K_3},$$

where K_4 is the dissociation constant of water, K_2 that of the acid, and K_3 that of the base, we see that the degree of hydrolysis can be calculated when the strengths of the acid and base are known, and that it may have the same value with very various relative values of K_2 and K_3, being greatest when both are low. Moreover, if the one or the other of the non-dissociated components is insoluble, it may happen that nearly the whole of the solute is hydrolysed. An instructive case, where the process of hydrolytic dissociation is visible, is that of mercuric acetate; a fresh solution is clear, but gradually becomes more and more turbid and red oxide is deposited.

The fact is sometimes overlooked that this process of hydrolysis in water rarely amounts to more than 3 to 5 per cent. of the total content of solute. When both acid and base are weak, as in aniline acetate, the hydrolysis may amount to 28 per cent. (Bayliss, 1909, 2, p. 359). But, as a rule, it is a small thing compared with electrolytic dissociation, and indeed is not always to be found when it might be expected. For example, it appears that sodium stearate is considerably hydrolysed, sodium palmitate is not. Congo-red is not so to any appreciable degree, neither is the sodium salt of caseinogen. The acids in these cases are insoluble in water, so that it is a matter of much difficulty to know *à priori* what are to be reckoned as strong acids.

We may now return to the consideration of the *phosphate system*.

In a solution of NaH_2PO_4, which has an acid reaction, the only source of $H^{\cdot}$ ions is the stage of dissociation numbered (4) in the list above. (2) must precede this, so that, combining the two, we have :—

$$NaH_2PO_4 = Na^{\cdot} + H^{\cdot} + HPO_4''.$$

In a solution of Na_2HPO_4 we have also HPO_4'' ions from (1) and (3) :—

$$Na_2HPO_4 = Na^{\cdot} + Na^{\cdot} + HPO_4''.$$

If we add Na_2HPO_4 to a solution of NaH_2PO_4, we add an excess of HPO_4'' ions. Therefore, since these solutions, as weak acids and bases, obey the law of mass action, we reverse the dissociation of equation (4)—

$$H_2PO_4' \rightleftarrows H^{\cdot} + HPO_4'',$$

and the $H^{\cdot}$ ion concentration of the acid phosphate is reduced.

Similarly, the alkalinity of a solution of Na_2HPO_4 is due to the OH' ions derived from hydrolysis of HPO_4'' ions, according to equation (6). Perhaps it would be more correctly expressed by saying that the HPO_4'' ion combines with $H^{\cdot}$ ions of water to form H_2PO_4' ions, in a way analogous to that in which acetic anions combine with hydrogen ions to form non-dissociated acetic acid. In any case the result is an excess of OH' ions. If, then, NaH_2PO_4 is added to Na_2HPO_4, the excess of H_2PO_4' ions throws back equation (6), and the alkalinity is reduced.

The mono-sodium phosphate, as a weak acid, gives off very few $H^{\cdot}$ and HPO_4'' ions by (2) and (4), so that a very small amount of the di-sodium salt, which, as a sodium salt, gives many HPO_4'' ions by (1) and (3), has considerable power of diminishing the acidity of the former. Again, the di-sodium salt as a weak base gives rise to very few OH' ions by (1), (3), and (6). Hence a very small amount of NaH_2PO_4, which, in its character as a sodium salt, dissociates with the production of many H_2PO_4' ions, diminishes considerably the hydroxyl ion concentration of the di-sodium salt by throwing back equation (6).

These considerations show that phosphate mixtures vary comparatively little from neutrality, even with considerable excess of the acid or alkaline constituent

For this reason they make useful standard mixtures for hydrogen ion concentrations not far removed from neutrality, as we shall see later.

The Bicarbonate System.—Similar considerations may be applied to the bicarbonate and carbon dioxide system. In actual dissociation the conditions are not so complex, since we have to deal with a dibasic acid instead of a tribasic one. On the other hand, there is a new complication added in the escape of CO_2 as a gas.

The equations of dissociation may be written thus, to correspond with those of the phosphates:—

$$(3)\ NaHCO_3 \rightleftarrows Na^{\cdot} + HCO_3'.$$
$$(4)\ H_2CO_3 \rightleftarrows H^{\cdot} + HCO_3'.$$
$$(5)\ H_2O \rightleftarrows H^{\cdot} + OH'.$$
$$(6)\ HCO_3' + H_2O \rightleftarrows H_2CO_3 + OH'.$$

Since carbonic acid, H_2CO_3, is a very weak acid, few hydrogen ions are formed by equation (4). Sodium bicarbonate, as a weak base, produces few hydroxyl ions, but, as a sodium salt, produces a considerable number of HCO_3' ions. Suppose that CO_2 is added to a mixture of bicarbonate and CO_2. H_2CO_3 is formed, and this increases the concentration of HCO_3' by dissociation. The result of this will be increase of non-dissociated $NaHCO_3$ by throwing back equation (3).

The way in which these facts work in the maintenance of moderate changes only in $H^{\cdot}$ ion concentration will best be seen by taking a numerical example. We must first, however, refer to the principle of ***isohydric solutions***. This states that, if two solutions have an ion in common and in the same concentration in both, no change in the concentration of this ion will take place when the solutions are mixed.

The dissociation constant of H_2CO_3 is 3×10^{-7}, hence—

$$(3 \times 10^{-7})\ (H_2CO_3) = (H^{\cdot})\ (HCO_3'),$$

and that of H_2PO_4' is 2×10^{-7}, according to Lawrence J. Henderson (1909, p. 269), hence—

$$(2 \times 10^{-7})\ (H_2PO_4') = (H^{\cdot})\ (HPO_4'').$$

Suppose that H_2CO_3 and $NaHCO_3$ are present together in a solution. From the low value of the dissociation constant of the former we may assume that the concentration of the non-dissociated H_2CO_3 is almost exactly the same as that of the dissolved CO_2; practically all the HCO_3' ions, therefore, come from the strongly dissociated $NaHCO_3$, and their concentration is proportional to it—that is, in decimolar concentration, about 0·8 of it, since this is the proportion dissociated. The dissociation of NaH_2PO_4 is also 0·8, and that of Na_2HPO_4, as regards $H^{\cdot}$ ion, is **0·64**.

We may write the above equations thus:—

$$(H^{\cdot}) = \frac{(H_2CO_3)}{(HCO_3')} \times (3 \times 10^{-7}) = \frac{(H_2PO_4')}{(HPO_4'')} \times (2 \times 10^{-7}),$$

and, if the salts are in decimolar concentration:—

$$(H^{\cdot}) = \frac{(H_2CO_3)}{0{\cdot}8(NaHCO_3)} \times (3 \times 10^{-7}) = \frac{0{\cdot}8(NaH_2PO_4)}{0{\cdot}64(Na_2HPO_4)} \times (2 \times 10^{-7}).$$

Hence, to obtain a hydrogen ion concentration of 1×10^{-7} (*i.e.*, neutrality at 24°)—

$$\frac{(H_2CO_3)}{(NaHCO_3)} = \frac{1}{3{\cdot}75} \text{ or } \frac{(NaH_2PO_4)}{(Na_2HPO_4)} = \frac{1}{2{\cdot}5},$$

an expression which gives the proportion of the constituents necessary for neutrality in a solution containing all four, or either pair, since they are isohydric. The absolute concentrations may vary so long as the ratios are kept constant, and the latter can only change if dissociation constants change.

For the sake of simplicity, we will take for further consideration the first

(CO_2) system, having a total concentration in CO_2 of decimolar strength, which corresponds very closely to that of blood. Let us see what change is necessary to raise the H· ion concentration from $0{\cdot}5 \times 10^{-7}$ to $1{\cdot}0 \times 10^{-7}$, keeping, for ease of calculation, the total CO_2 constant by dilution. In the manner described above we have—

$$0{\cdot}5 \times 10^{-7} = (3 \times 10^{-7}) \times \frac{(H_2CO_3)}{0{\cdot}8(NaHCO_3)} \text{ or } \frac{(H_2CO_3)}{(NaHCO_3)} = \frac{1}{7{\cdot}5},$$

i.e., $(H_2CO_3) = 0{\cdot}012$ molar and $(NaHCO_3) = 0{\cdot}088$ molar, together $0{\cdot}1$ molar.

From the previous calculation, we have, for 1×10^{-7}, a value for the ratio of $\frac{1}{3{\cdot}75}$, so that the concentration of $NaHCO_3$ in this case must be $0{\cdot}046$ molar. The difference between this and the value for $0{\cdot}5 \times 10^{-7}$ is $0{\cdot}088 - 0{\cdot}046 = 0{\cdot}042$ gram-molecules of $NaHCO_3$ or CO_2. This shows that nearly half as much CO_2 as the bicarbonate present is required in order to produce a change of hydrogen ion so small as that from $0{\cdot}5 \times 10^{-7}$ to 1×10^{-7}, which is about what would be produced by the addition of 0·001 gram-molecule of hydrochloric acid to 10,000 litres of water.

A similar calculation can be made of the amount of bicarbonate required to reduce the hydrogen ion concentration from $0{\cdot}5 \times 10^{-7}$ to $0{\cdot}2 \times 10^{-7}$. Thus:—

$$0{\cdot}2 \times 10^{-7} = \frac{(H_2CO_3)}{0{\cdot}8(NaHCO_3)} \times (3 \times 10^{-7}) \text{ or } \frac{1}{18{\cdot}7}.$$

That is, 0·228 molar in bicarbonate; and $0{\cdot}228 - 0{\cdot}088 = 0{\cdot}140$ molar, or nearly twice as much, alkaline salt must be added as that originally present.

The phosphate equilibrium can be treated in the same way, so that we can understand the great capacity of blood and cells to preserve an almost complete neutrality.

The results of the preceding calculations may be further realised in the following way. In a bicarbonate system with a constant pressure of CO_2, in order to change an acidity of 0·0000002 molar into an alkalinity of the same value, an extremely small change, it is necessary to add a volume of decinormal sodium hydroxide nearly equal in volume to the solution itself. On account of the importance of the question, another example may be given (see L. J. Henderson, 1913, pp. 147-152). Consider 1 kg. of CO_2 dissolved in 100 litres of water and that sodium hydroxide is added in quantities of 50 g. at a time. Before any addition, the hydrogen ion concentration is about 10^{-4}, or about 1,000 times that at neutrality. The addition of 50 g. of NaOH reduces this to 50 times that at neutrality. After the addition of 200 g. more, the H· ion concentration is only 10^{-6}, merely 10 times that at neutrality, although there are still present 682 g. of free CO_2. An acidity of this order is produced by the addition of only 0·004 g. of hydrochloric acid to 100 litres of pure water. We can continue to add NaOH without causing any change, more than just perceptible, until 450 g. more have been added. When 700 g. in all have been added, the reaction is practically that of pure water, and a further 50 g. may be added without any greater change in the H· ion concentration than from $0{\cdot}9 \times 10^{-7}$ to $0{\cdot}6 \times 10^{-7}$, and in the OH′ ion concentration from $1{\cdot}1 \times 10^{-7}$ to $1{\cdot}7 \times 10^{-7}$, although in pure water one ten-thousandth part of the amount would reduce the H· ion concentration from $1{\cdot}1 \times 10^{-7}$ to $0{\cdot}1 \times 10^{-7}$ and raise that of the OH′ ions from $1{\cdot}1 \times 10^{-7}$, to 12×10^{-7}. The same amount (50 g.) added to pure water would raise the OH′ ion concentration to $120{,}000 \times 10^{-7}$.

Suppose now that we take a case which is analogous to that of the blood of air-breathing animals. The state of affairs will be found to be still more striking. In the experiment described by L. J. Henderson (1913, pp. 149-151) we take a solution of 1 kg. of sodium bicarbonate in 100 litres of water and allow it to attain equilibrium with an unlimited atmosphere containing 1 g. of CO_2 per litre. Let hydrochloric acid be added in small portions at a time, constantly shaking the solution so that there shall always be equilibrium with the CO_2 in the gas phase. Further, let the temperature be such that

the absorption coefficient of CO_2 is unity, that is, about 17°. Then the stages will be about as given in the following table:—

HCl.	$H_2CO_3 : NaHCO_3$	(H·) N ×	(OH′) N ×	Acidity Relative to Neutrality.	Alkalinity Relative to Neutrality.
0	2·27 : 11·9	0·000000057	0·000000176	0·57	1·76
10	2·27 : 11·5	0·000000059	0·000000170	0·59	1·70
50	2·27 : 10·0	0·000000068	0·000000147	0·68	1·47
100	2·27 : 8·2	0·000000083	0·000000120	0·83	1·20
150	2·27 : 6·3	0·000000108	0·000000093	1·08	0·93
200	2·27 : 4·4	0·000000154	0·000000065	1·54	0·65
250	2·27 : 2·6	0·00000026	0·000000039	2·6	0·39
300	2·27 : 0·68	0·0000010	0·000000010	10	0·10
310	2·27 : 0·31	0·0000022	0·0000000045	22	0·045
318	∞	0·00026	0·00000000039	260	0·0039
320	...	0·00045	0·00000000022	450	0·0022
330	...	0·0027	0·000000000037	2700	0·00037

Until nearly 250 g. of hydrochloric acid have been added, neither the acidity nor the alkalinity is greater than twice that of a perfectly neutral solution. The cause of this constancy is simple enough. At the beginning the free CO_2 of the solution is in equilibrium with that of the gas phase. Accordingly when hydrochloric acid is added and reacts to form sodium chloride and more CO_2, the whole of the latter escapes to the gas phase and the total amount of acid is what it was before, viz., saturation with CO_2 at a partial pressure of 1 g. per litre of air, since all the hydrochloric acid has combined with the bicarbonate. Thus the concentration of the alkaline salt (bicarbonate) is diminished, but there is no increase of free acid. Not until all the bicarbonate is decomposed does the hydrochloric acid begin to show its effect, and then the addition of 2 g. causes nearly as much rise in acidity as the previous 318 g. had done, or about 200 times the rise caused by 100 times the amount at the first stage of the experiment.

A remarkable fact was noticed by Henderson (1908, p. 176) in comparing the relative amounts of alkali necessary to produce a given change in the H· ion concentration, as shown by indicators, in the cases of various weak acids. With the single exception of hydrogen sulphide, it was found that *NaH_2PO_4 and H_2CO_3 required the largest quantities.* Acids both weaker and stronger than these required very much less, there being a large step between the three mentioned and the next in the series.

The "Fitness" of Carbon Dioxide.—It will probably not have escaped the reader that, as is insisted upon by L. J. Henderson (1913), it is a remarkable fact that it should be carbon dioxide, the universal product of oxidation in the living organism, that is the most efficient regulator of neutrality. Of course it is clear that organisms would not have been able to develop to their present degree of perfection without some mechanism of this kind, and that it is in adaptation to a system in which carbon compounds play the chief part that their mechanisms have been evolved. None the less it is calculated to excite a certain amount of wonder that the element carbon, which is, as pointed out above (page 41), so peculiarly adapted for the formation of a great variety of complex compounds, should also include amongst these an acid with the properties which carbon dioxide alone, with the exception of hydrogen sulphide, possesses. Especially is this so when we remember that there is no reason to suppose that this property is necessarily connected with the other properties of carbon. In the next chapter we shall see that similar remarks apply with even more force to the case of water.

In this connection we may call to mind what Parker (1913, 1) points out, namely, that many apparent adaptations are not really such. That a person who faints falls with muscles limp is appropriate for recovery, and it is also the safest way to fall, but these conditions are the direct consequence of the faint, and that they are advantageous is purely incidental; they might, in fact, have been the opposite, but they would happen, notwithstanding. Parker holds that the majority of animal reactions are, probably, neither of advantage nor disadvantage, in any notable degree, to the life of the individual, but dependent on the con-

struction, physical and chemical, of the given organism. At the same time, he points out that there are real adaptations. The capacity of an individual to react appropriately to his environment has been brought about by the elimination of myriads of individuals who failed to do so. Adaptation has been regarded as a sort of transcendental property of organisms, an entelechy, allied to intelligence. But, as Parker remarks, what do we really mean by intelligence other than "that aggregate of nervous states and actions which is our chief means of adaptation"? so that the proper understanding of adaptive reactions implies that of intelligence, and conversely. The introduction of such notions as entelechies consists, practically, in argument in a circle and is rather calculated to retard progress by apparent explanation, when what is really wanted is research into the very questions which they pretend to answer. "The details of animal reactions are then, in the main, free from adaptive restraint and their diversity is dependent chiefly upon the fluctuating momentary condition of the animal body; further, the main outlines of animal reactions are adaptive, but are not to be explained by the assumption of something like intelligence."

An interesting case of apparent complex adaptation is that of the mollusc *Onchidium* (Crozier and Arey, 1919), the mechanism of whose behaviour is found to depend on heliotropic reaction, together with inhibition from the rocks over which it creeps.

The effect of *Rise of Temperature* on hydrogen ion concentration is of some importance. In a previous page the large temperature coefficient of electrolytic dissociation of water was referred to in another connection, as being of the order of those regarded as characteristic of chemical reactions. That of sodium salts, on the other hand, is the very low one of salts in general, which do not obey the Ostwald "dilution law." On mixtures of bicarbonate and CO_2 the net effect of a rise of temperature will be to increase the alkalinity, since the dissociation of water will be increased more than that of the bicarbonate. Thus water at 18° has a dissociation constant of $0{\cdot}64 \times 10^{-14}$, *i.e.*,

$$(C)_{OH} \times (C)_{H} = 0{\cdot}64 \times 10^{-14}.$$

A solution of sodium bicarbonate and CO_2, which has, at 18°, a hydrogen ion concentration of $0{\cdot}30 \times 10^{-7}$, has accordingly a hydroxyl ion concentration of

$$\frac{0{\cdot}64 \times 10^{-14}}{0{\cdot}30 \times 10^{-7}} = 2{\cdot}1 \times 10^{-7},$$

since the product of $(C)_{OH'}$ and $(C)_{H^{\cdot}}$ is constant in all solutions in water at the same temperature.

At 42°, the hydrogen ion concentration of the bicarbonate mixture has risen only to $0{\cdot}42 \times 10^{-7}$, owing to its low temperature coefficient, whereas the dissociation constant of water has become $3{\cdot}76 \times 10^{-14}$ (Kohlrausch and Heydweiller, 1894, p. 209). Hence the hydroxyl ion concentration has risen to

$$\frac{3{\cdot}76 \times 10^{-14}}{0{\cdot}43 \times 10^{-7}} = 9{\cdot}0 \times 10^{-7}$$

or 4·3 times as great as at 18°.

The *proteins* present in blood and tissues play little, if any, part in neutralising acid and alkali within the limits possible to occur in the living organism, namely, 10^{-4} and 10^{-10} normal in $H^{\cdot}$ ion. This appears to be due to the existence of proteins between these limits in the form of internal ammonium salts, similar to the betaines. Thus:

Ordinary betaine is $CH_2\!\left\langle\begin{matrix} N(CH_3)_3 \\ COO \end{matrix}\right.$ Glycine is $CH_2\!\left\langle\begin{matrix} NH_3 \\ COO, \end{matrix}\right.$

and proteins are $R\!\left\langle\begin{matrix} NH_3 \\ COO \end{matrix}\right.$ not $R\!\left\langle\begin{matrix} NH_2 \\ COOH \end{matrix}\right.$ nor $R\!\left\langle\begin{matrix} NH_3OH \\ COOH. \end{matrix}\right.$

The ring is broken, in order to produce the last form, only by an acid reaction above 10^{-4} N or an alkaline one of 10^{-10} N in $H^{\cdot}$ ion. Combination with acid or base can then take place (see Bayliss, 1919, 2, p. 173). Solutions of pure proteins possess scarcely any electrical conductivity. Further facts in relation to amino-acids will be found on page 220.

The Reaction of Blood.—By the most sensitive methods available, the hydrogen ion concentration of blood at 38° is found to be $0{\cdot}4 \times 10^{-7}$ and the corresponding OH' ion concentration, $7{\cdot}2 \times 10^{-7}$ molar. So that it is just on the alkaline side of neutrality. At room temperature, the alkalinity would be somewhat less, owing to the increase of OH' ion concentration in CO_2—bicarbonate systems with rise of temperature, as described above. Direct measurements of the effect of temperature on such systems, in moderately concentrated form, have shown about four times as great an alkalinity at 38° as at 18°. This is dependent on

the fact that the electrolytic dissociation of water rises more quickly with temperature than that of sodium bicarbonate does.

The reaction of blood can be determined by using neutral red as indicator (Bayliss, 1919, 2, p. 162).

Protoplasm possesses, in the phosphates present therein, an efficient mechanism for avoiding any considerable change in reaction. All the phosphate must be converted into the acid salt before the hydrogen ion concentration can rise beyond that due to this salt, or into the alkaline salt before the alkalinity can become greater than that of solutions of Na_2HPO_4.

"*Buffers.*"—The effect of such substances as bicarbonates, phosphates, amino-acids, etc., in "soaking up," as it were, excess of hydrogen or hydroxyl ions was compared by Fernbach and Hubert (1900, p. 295) to that of "tampons." Sörensen (1909) adopted the word and, in the translation of his paper into German, it was rendered "Puffer" and thence into English as "Buffer." This latter word does not seem to me to be a very descriptive one nor to convey correctly the meaning of the original "tampon." A railway buffer does not absorb the engine itself, as the substances referred to absorb ions. A word more suggestive of a sponge would probably be better, but is not easy to find.

The Practical Use of Phosphate Mixtures.—In certain cases it is of much importance to be able to obtain a solution of a definite but very small concentration in hydrogen ions, as also to possess the means of maintaining constant this value in a system in which chemical changes, sensitive to change of reaction, are going on. Such cases are the action of enzymes, or the solutions used for perfusion of living organs. The bicarbonates are the most appropriate for the latter purpose. For the making of standard solutions as well as for use with enzymes, the phosphate systems are most valuable.

These phosphate mixtures are most readily prepared by the addition of standard sodium hydroxide in different proportions to standard phosphoric acid solution. The following table, from the paper by Prideaux (1911) with additions, will be found useful:—

C.c. NaOH (Molar) to 10 c.c. of Molar H_3PO_4 in 100 c.c. of Water.	H· ion.	Colour to Neutral Red.	Colour to Other Indicators.
10·0	10^{-4}	Crimson	Red to methyl-orange.
10·6	10^{-5}	Crimson	Orange to methyl-orange, red to methyl-red.
11·5	10^{-6}	Trace of red	Orange to methyl-red.
13·0	$10^{-6\cdot4}$	¾ crimson, ¼ red	Yellow-orange to methyl-red.
15·5	$10^{-6\cdot7}$	½ crimson, ½ red	Yellow to methyl-red.
16·0	$10^{-6\cdot8}$	¼ crimson, ¾ red	
16·5	$10^{-6\cdot9}$	Red	
17·2	10^{-7}	Trace of orange	
17·7	$10^{-7\cdot07}$	¼ orange, ¾ red	
18·0	$10^{-7\cdot2}$	½ orange, ½ red	
18·5	$10^{-7\cdot4}$	¾ orange, ¼ red	
19·2	$10^{-7\cdot7}$	Orange	
19·6	10^{-8}	Trace more yellow	
20·0	10^{-9}	¾ orange, ¼ yellow	Colourless to phenolphthalein.
20·4	10^{-10}	½ orange, ½ yellow	Faint red to phenolphthalein.
21·0	$10^{-10\cdot5}$	¼ orange, ¾ yellow	Red to phenolphthalein, colourless to thymolphthalein.
22·6	10^{-11}	Yellow	Blue to thymolphthalein, yellow to tropæolin O.
28·5	10^{-12}	Yellow	Orange to tropæolin O.

From Sörensen's work it would appear that the H· ion exponent of these solutions may require increasing by 0·2. Thus, a mixture of 10 parts of acid with 17·2 of alkali may have a value of $10^{-7\cdot2}$ instead of 10^{-7}. (See Prideaux, 1919.) The curve would thus require raising by two divisions of the scale.

The equation by which any other required hydrogen ion concentration can be

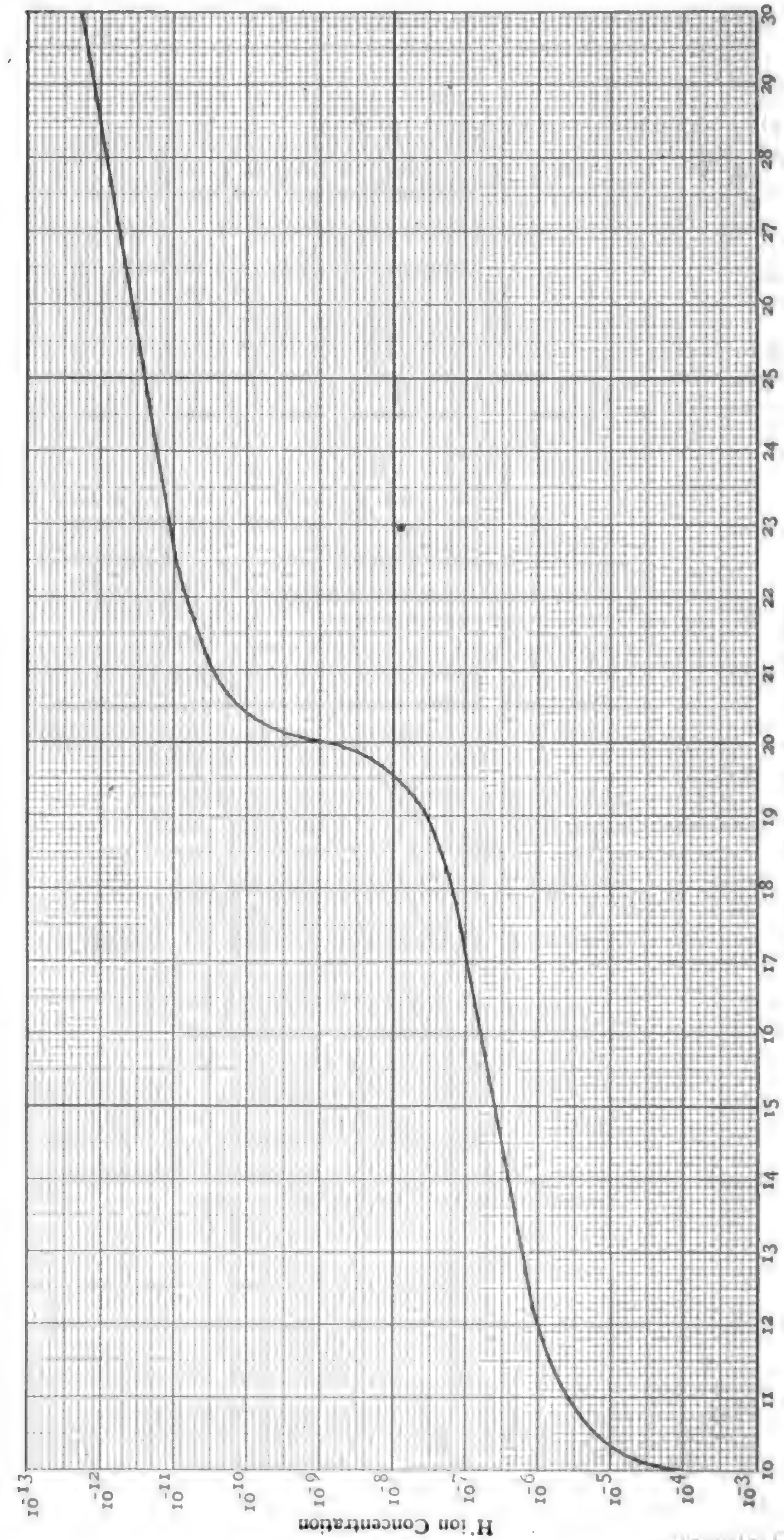

FIG. 58. (1) CURVES FOR PREPARING PHOSPHATE SOLUTIONS OF DEFINITE HYDROGEN ION CONCENTRATIONS.

(Prideaux, 1911.)

obtained will be found in the paper by Prideaux on p. 125. The values may also be read on the curves of Fig. 58, copied from this paper. The abscissæ give the number of c.c. of molar sodium hydroxide to be added to 10 c.c. of molar phosphoric acid to make 100 c.c. of solution, in order that we may have a hydrogen ion concentration of the ordinate selected. The figure on page 205 is the steeper parts of the complete curve drawn on a larger scale, so that greater accuracy may be attained by preparing a larger volume of the solution, say a litre.

To illustrate the use of the curve:—Suppose that a solution of the optimal acidity for emulsin is required. This is, according to Vulquin (1911), 5×10^{-6} in H· ions. The exponent of 10 which we require is log. 5 minus 6 times log. 10 = −5·30. Corresponding to this ordinate in the table we find 10·8; we must, therefore, add 10·8 c.c. of molar NaOH to 10 c.c. of molar phosphoric acid and dilute to 100 c.c. For the use of other mixtures, see Prideaux (1916).

PHYSIOLOGICAL SALINE SOLUTIONS

In the case of organisms whose cells are unprotected by a resistant envelope, it has been already pointed out that the solutions which bathe them must have the same osmotic pressure as the cell contents. Otherwise the cell will contract or expand, by the loss or gain of water, until its osmotic

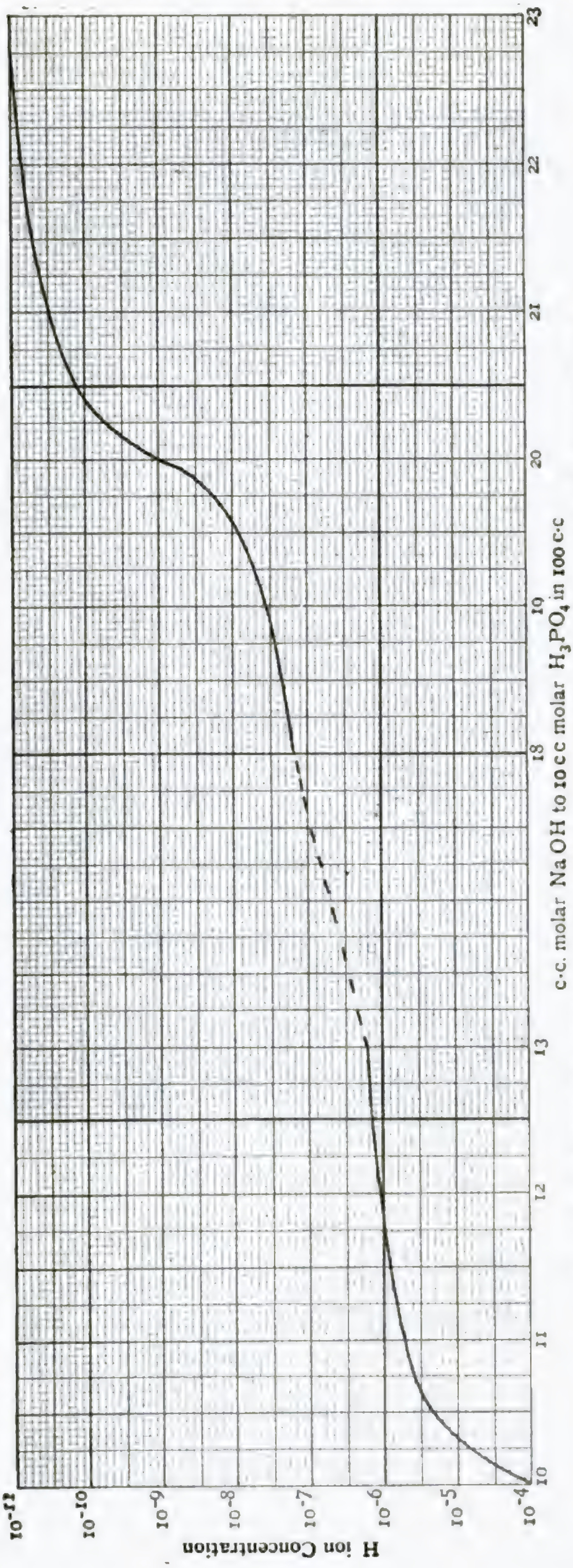

FIG. 58. (2) CURVE FOR PREPARING PHOSPHATE SOLUTIONS OF DEFINITE HYDROGEN ION CONCENTRATION.

(Prideaux, 1911.)

pressure is equal to that of the surrounding solution. If this is impossible, the cell will be destroyed. In any case, the concentration or dilution of the contents will seriously impair their functional activity. These remarks apply especially to animal cells and it is in the investigation of these that the necessity for solutions of equal osmotic pressure to themselves is met with. It is often required to replace the blood or other solution with which the cells are normally in contact by some artificial

Sydney Ringer

FIG. 59. PORTRAIT OF SYDNEY RINGER.

(From the Obituary Notice in *Proc. R.S.*, 84B.)

solution, whose composition is known and can be modified at will. The blood plasma of the same species of animal has been supposed indispensable for the growth of excised tissues in the work of Ross Harrison, and others. (But see Thomson, 1914.) But if an efficient substitute can be found for other purposes, the advantages are obvious, and indeed Lewis and Lewis (1911) have grown tissues in artificial media.

It might be supposed that a solution of any substance, so long as it is not actually toxic, would suffice, provided that the cell membrane is impermeable to the solute and it is present in the correct concentration. Sodium chloride, as one of the salts present in all animal fluids, was selected at an early date and was found to serve well for the histological examination of fresh tissues or for the dilution of blood without causing changes in volume in the corpuscles. But when used by Ringer (1880-82, 1882-83, 1 and 2) for continuous perfusion of the heart of the frog, it was found unable to maintain the normal beat. The work of Ringer on this question is fundamental and enabled a satisfactory perfusion fluid to be made. Although this solution is used everywhere and known as "Ringer's Solution," its origin is apt to be forgotten, so that it is necessary to give a brief account of the researches which led to its composition being established. A portrait of Sydney Ringer himself will be found in Fig. 59.

When the heart was perfused with a solution of sodium chloride in distilled water, isotonic with the blood, that is, 0·75 per cent., the beats gradually diminished in extent and ultimately ceased (1882-83, 1, p. 31), as shown in Fig. 60. The excitability to electrical stimuli also disappeared.

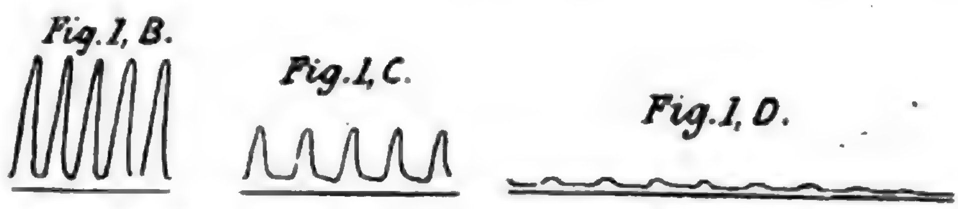

FIG. 60. THE ACTION ON THE FROG'S HEART OF A SOLUTION CONTAINING SODIUM CHLORIDE ONLY.

1, *B*, Tracing obtained eight minutes after replacing blood by pure sodium chloride, 0·75 per cent.
1, *C*, Six minutes later.
1, *D*, After another four minutes' action.

(Ringer, 1882-83, 1, p. 33.)

We may note here that subsequent work has shown that this action of pure sodium chloride is not only due to the want of some essential salt, but also to a toxic action of the Na· ions, similar to, but less marked than that of potassium ions to be described presently. Clark (1913, 2, p. 77) finds indeed that the ordinary Ringer solution is improved when a part of the sodium chloride is replaced by isotonic cane-sugar, and Abel (1914), to avoid œdema in his "vivi-diffusion" experiments, found it advisable to reduce the sodium chloride to 0·6 per cent.

FIG. 61. The effect of adding 5 c.c. of 0·25 per cent. calcium chloride solution to 100 c.c. of the pure sodium chloride solution. The heart-beats, which had ceased under the pure sodium chloride, became spontaneous after one artificial stimulus, but the diastole was prolonged so that the beats fused. (Ringer, 1882-83, 1, p. 33.)

To proceed with the experiments of Ringer, it was found that, if calcium chloride were added to the pure sodium chloride solution when the heart had ceased to beat, the excitability to stimuli returned and was soon followed by spontaneous beats, but that the relaxation was imperfect and delayed, so that there was a tendency to a tonic, systolic state (Fig. 61).

This condition is seen, although less markedly, in the figures of Plate 2 of the first paper (1880-82), where saline solutions made with tap water, containing calcium, were used.

It was next discovered (1882-83, 1, p. 35) that a trace of a potassium salt (1 c.c. of 1 per cent. KCl to 100 c.c. of the solution of sodium chloride in tap water) abolished this tonic action of calcium, without depriving it of the power of neutral-

ising the injurious effect of the pure sodium chloride (Fig. 62). A solution capable of maintaining the heart beat at a satisfactory height for a considerable time was thus obtained, but, from what has been said in the previous pages of the present chapter, it is not surprising to find, as Ringer himself did, that the addition of a small amount of sodium bicarbonate was beneficial. The investigator himself pointed out that this addition had the effect of producing a slight alkalinity similar to that of the blood, and of neutralising acid produced in the contractions of the heart muscle (see 1882-83, 2, p. 223). The amount used was 5 c.c. of a 1 per cent. solution to 100 c.c. of the circulating fluid.

Although the electrolytic dissociation theory was unknown at the time these experiments were made, it was clearly recognised by Ringer that the effects of calcium and potassium salts were due to the calcium and potassium components of the salts added. He himself used indifferently carbonate, sulphate, phosphate and chloride of calcium.

In view of the fundamental importance of these facts, the simplest way of demonstrating

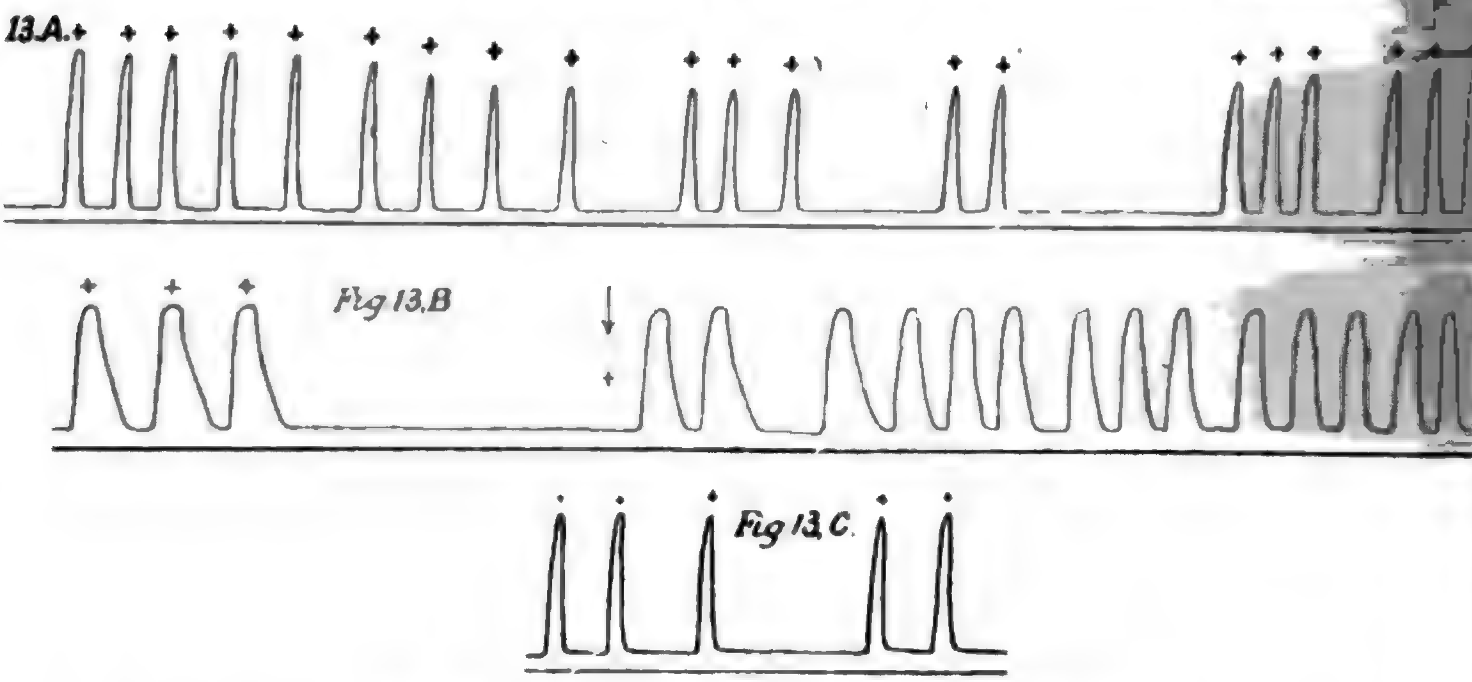

FIG. 62. ANTAGONISM OF CALCIUM AND POTASSIUM.

13.A, Normal beats.
13.B, Effect of adding calcium. The first three beats show the prolongation of the systole.
At the arrow, 3 minims of 1 per cent. potassium chloride were added to the solution. The calcium effect is partially abolished.
13.C, Addition of a further 2 minims of potassium chloride solution. The beat becomes quite normal.

(Ringer, 1882-83, 1.)

them may be described. The heart of the frog or the tortoise is tied on to a cannula inserted into the ventricle through the auricle by the method of Symes (1911). The way in which the effect of different electrolytes can be shown will best be understood from the description of an actual experiment. A tortoise heart was used and a tracing taken, by a lever attached to the apex of the ventricle, before any perfusion fluid was introduced (Fig. 63). The beats were small, as frequently happens, *a*. Perfusion was then commenced with a solution containing 0·75 per cent. sodium chloride and 0·01 per cent. sodium bicarbonate, *b*. The beats were not improved, and would probably have ceased, if the perfusion with this solution had been continued. Portions of the tracing are omitted for want of space. At *c*, a solution consisting of 100 c.c. of the previous one, to which 3 c.c. of decimolar calcium chloride had been added, was perfused. This contained a slight excess of calcium above the normal one. An immediate improvement is to be noticed, but relaxation is incomplete, as shown by the gradual rise in the level of the diastolic position. 6 c.c. of decimolar potassium chloride were then added to the solution already containing sodium and calcium. The tonic action of the calcium was removed, and, after a minute or two, the tracing *d* was obtained, showing a regular, powerful beat, which would have continued for a long time. At *e*, the solution containing sodium alone is returned to; the small irregular beat reappears. At *f*, potassium chloride, in the same proportion as before, is added. No improvement in the beat results, but the characteristic relaxing effect of potassium in the fall of the diastolic position is observed. At *g*, calcium chloride is added to the mixture in the same proportion as before, and we see the powerful regular beat produced by the normal Ringer solution, containing sodium, potassium, and calcium. In order to observe the effect of calcium in a more marked way, at *h*, another

3 c.c. of the calcium chloride solution were added, and a further 3 c.c. before each step in the tracing. We note the increase of tonic contraction brought about by each addition. At *k*, potassium chloride was added, but, although it diminished the systolic condition, the size of the beat was rather decreased; in fact, the antagonism is not complete when there is excess of either calcium or potassium much beyond the normal proportion. Finally, at *l*, normal Ringer solution was perfused.

It is a remarkable fact that the proportion of sodium, potassium, and calcium ions in *sea water* is almost identical with that found by Ringer to be the best for maintaining the beat of the heart, although the total concentration in sea water is higher. Magnesium salts, however, are present in sea water, in addition to those mentioned. The presence of magnesium does not appear to be necessary in an artificial physiological saline solution, although Neukirch (1912) found that the contractions of the excised intestine of the rabbit were more regular when magnesium was present.

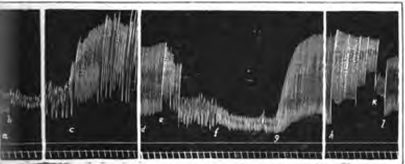

FIG. 63. ACTION OF ELECTROLYTES ON THE HEART OF THE TORTOISE.

a, Excised heart before perfusion.
b, Perfusion with sodium chloride, 0·75 per cent., sodium bicarbonate, 0·01 per cent.
c, Addition of 3 c.c. of 0·1 molar calcium chloride to 100 c.c.
d, Potassium chloride added, 6 c.c. of 0·1 molar to 100 c.c. of the mixture containing sodium and calcium salts.
e, Pure sodium chloride again.
f, Potassium chloride, 6 c.c. to 100.
g, Calcium chloride added.
h, 3 c.c. calcium chloride added before each step in the tracing.
k, More potassium chloride added, in amount corresponding to the calcium chloride present. The tonic action of the calcium is partly abolished, but the beat does not return to its normal height.
l. Normal Ringer's solution.

b–*c* and *e*–*f* = Na alone, *c*–*d* = Na + Ca, *d*–*e* and *g*–*h* = Na + Ca + K, *f*–*g* = Na + K.

If we look at the relative proportions of these ions in blood serum and in sea water, viz. :—

	Blood Serum.	Sea Water.
Na	100	100
Ca	2·58	3·84
K	6·69	3·66

(Macallum, 1910, p. 603)

we notice that the proportion of calcium to sodium is very similar and that of potassium to sodium is not very far different in the two solutions, but there is a great excess of magnesium in sea water. The probable reasons for the divergences will be seen presently. Bunge (1894, p. 120) made the suggestion that the high content in sodium chloride of the blood of land vertebrates in comparison with that of their surroundings is an inheritance from marine

ancestors who lived in a solution fairly rich in this salt. Macallum (1903, p. 234), struck by the similarity between the proportion of potassium and calcium to sodium in the blood plasma of vertebrates and that in sea water, was led, independently, to advocate the same view.

The ocean, ever since the first condensation of water on the earth's surface, has been continually receiving salts by dissolving them from its bed and from the contents of the rivers flowing into it. Since the salts are left behind on evaporation, while water vapour is continually rising to form new rivers, which wash away more constituents of the land, it is easy to understand why the total concentration of salts in sea water is, at the present time, so much higher than that which it was at the time when the ancestors of the land vertebrates left it.

It is generally believed that life began in the ocean and continued in it alone until the close of the Cambrian period. When vertebrates with a closed circulatory system took to the land, they took with them a blood of the same composition, as regards salt, as the sea water which they left behind.

The Cambrian period was an extremely long one, judging by the thickness of the deposits, amounting to 40,000 feet in British Columbia, and 12,000 feet in Wales, although it varies in different places. It is to be expected, therefore, that the protoplasm would have become adjusted to the salts of the sea during this long period, and that mechanisms would have been produced to maintain the concentration in the blood at the same value. These mechanisms still continue to act since life on land began.

If this view is correct, the salt composition of the blood represents that of the ocean in the early Cambrian period. As regards the proportion of calcium and potassium in sea water, Macallum points out that, at the present time, the concentrations of these two salts is scarcely changing at all. Calcium is being continually removed by living animals for the formation of bones, coral, shells, etc., as fast as it is supplied by the rivers. Potassium, since the great development on land of plant life, with its comparatively large content in this element, is supplied by the rivers in much less quantity than it was in early geologic times. The chief difficulty is the magnesium, which is present now in so much greater ratio to sodium in sea water than it is in blood plasma. According to Macallum, the reason is that the magnesium content of sea water is still slowly increasing, so that "in the pre-Cambrian oceans it must have been very small, not perhaps as low as it is in blood plasma, for in the latter the magnesium would only represent the proportion of an earlier period than that in which the circulation became closed, as the tissues would only reproduce the proportion which had by long accommodation become fixed in them. Even the organisms which live in the sea to-day, whose ancestral forms have lived in the sea since the Cambrian, do not take up the magnesium from the sea water in the full proportion which it has in the latter" (1904, p. 8 of reprint). Chemical changes by which magnesium chloride in the primeval ocean became precipitated as magnesia must also be taken into account (1904, p. 12).

A further interesting question concerns the salts of the cells themselves, a more difficult problem; but, as Macallum puts it (1904, p. 9 of reprint), "If the blood plasma of vertebrates, because of the forces of heredity, reproduce the proportions which obtained in pre-Cambrian oceans, why should not the cells of the tissues, because of the same forces, reproduce in themselves the proportions which obtained in sea water of a much earlier geological period?"

There are different questions involved in the discussion of this problem, the consideration of which would lead us too far. The reader interested may refer to the paper quoted, and to a further one on the salts of the blood (1910).

Whatever may be the final decision on the question, the fact remains that sea water, diluted to the same osmotic pressure as the blood, is a very effective physiological solution, although the amount of magnesium is unnecessarily great.

Returning to the preparation of such solutions for experimental use, it is found

that *Ringer's solution* of the following composition is the most satisfactory for the heart of the frog:—

$NaCl$	0·65	NaH_2PO_4	0·001
KCl	0·014	(Glucose)	(0·2)
$CaCl_2$	0·012	Water	to 100
$NaHCO_3$	0·02		

This solution has a hydrogen ion concentration of $10^{-8\cdot3}$.

The osmotic pressure of the blood of warm-blooded vertebrates is higher than that of the frog, so that the concentration of the salts must be slightly raised. For the isolated mammalian heart, Locke (1900) found the following composition to serve well:—

$NaCl$	0·9	$NaHCO_3$	0·01-0·03
KCl	0·042	(Glucose)	(0·1-0·25)
$CaCl_2$	0·024	Water	to 100

The addition of glucose is of advantage as a source of energy, unless there is objection to its presence for other reasons. For mammals, this solution must be thoroughly oxygenated, conveniently by blowing oxygen from a cylinder of compressed gas through a Berkefeld filter immersed in the solution, as suggested by Keith Lucas. The solution used by Locke should be called *Ringer-Locke's solution*.

Tyrode (1910) adds a small amount of a salt of magnesium and bicarbonate, obtaining the following solution:—

$NaCl$	0·8	NaH_2PO_4	0·005
KCl	0·02	$NaHCO_3$	0.1
$CaCl_2$	0·02	Glucose	0·1
$MgCl_2$	0·01	Water	to 100

This solution is a good one for the intestine of the rabbit, but the addition of magnesium does not appear to be of any advantage for the heart, as pointed out by Locke (1900). The last solution may be called *Ringer-Tyrode's solution*.

The addition of bicarbonate is of value in assuring that the solution shall be only just on the alkaline side of neutrality, while possessing the capacity of neutralising acid products formed by the metabolism of the organ perfused (see p. 199).

The work of Clark (1913, 2) on the heart of the frog has shown that continuous perfusion with renewed supplies of a pure saline solution removes some important constituent from the cells; thus, a small volume of Ringer's solution, which has been repeatedly circulated through a heart, is capable of reviving another heart, whose beats are reduced owing to continuous perfusion (Fig. 64). The substance in question seems to be of a lipoid nature, since it has considerable power of lowering the surface tension of water, and a substance having a similar action can be extracted by ether from the residue left on evaporation of blood serum. The residue left on evaporation of the ether has a great effect in causing recovery of a heart which has become "hypodynamic" from prolonged perfusion with Ringer's solution (Fig. 65). This power is also present in lecithin. The interaction of calcium is necessary for the effect, which seems to be due to a change in the colloidal constitution of the cell membrane, which, as we have seen, undoubtedly contains a large proportion of lipoids. Cushny and Gunn (1913) show that the heart reacts abnormally to serum after perfusion with saline solutions.

Fig. 64.

A, Frog heart after perfusion for four hours with Ringer's solution. At the gaps, where 12' is marked, 2 c.c. of Ringer's solution, which had circulated for twelve hours backwards and forwards through another heart, were added to the 2 c.c. already circulating. The result shows that some important substance is removed from the muscle by continuous perfusion with saline solutions.

(Clark, 1913, 2, p. 98.)

Antagonism of Salts.—In these physiological saline solutions we see how one salt alone is unable to preserve the excitability of living tissues, and we are reminded of the similar phenomena in the effect of salts on the permeability of the cell membrane. In this latter case we found that, as a rule, the action of a single salt is to cause a loss of the semi-permeable properties of the membrane, and it may well be that we have to do with related phenomena in the behaviour of cells to perfusion fluids.

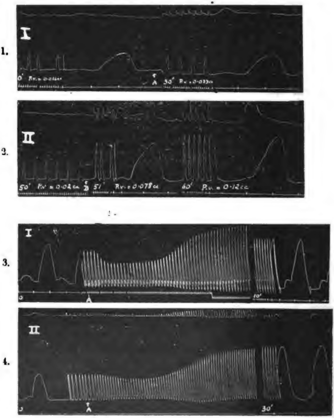

FIG. 65. EFFECT OF LIPOID CONSTITUENTS OF SERUM ON HYPODYNAMIC HEART.

1. Beats of heart rendered hypodynamic by prolonged perfusion with Ringer's solution. At *A* the constituents of serum which are insoluble in alcohol were added; there is no effect. The pulse volume (*P.v.*) increases merely from 0·026 to 0·033 c.c.
2. At *B* marked effect of the alcohol-soluble constituents of serum. Pulse volume increased in 60' from 0·02 c.c. to 0·12 c.c.
3. Effect of ether extract of dry residue of alcoholic extract of serum, introduced at *A*.
4 Similar effect of sodium soap of the above ether extract.

(Clark, 1913, 2, p. 94.)

The way in which one salt is able to neutralise the toxic properties of another is, as yet, by no means clear. Loeb (1903) showed that a marine Gammarus dies in half an hour if transferred to cane-sugar or pure sodium chloride isotonic with

sea water, in fact, just as quickly as if placed in distilled water. The addition of either potassium chloride or of calcium chloride alone to distilled water or to cane-sugar does not improve it. In solutions containing sodium chloride plus either potassium or calcium chloride, the animals die also as soon as in pure water. Only in a solution containing $Na^{\cdot}$, $K^{\cdot}$ and $Ca^{\cdot\cdot}$ ions in the proportion and concentration in which they exist in sea water are the animals able to remain alive. It seems clear that the normal semi-permeability of the colloidal constituents of the cell membrane is only kept intact when these three salts are present. Perhaps investigations on the physical properties of proteins and lipoids, as well as of other colloidal systems under the influence of these salts, separately and together, would throw light on the question. Loeb and Wasteneys (1915) showed that sodium chloride increases permeability.

A similar set of phenomena have been investigated by Loeb and Wasteneys (1911) in the case of the fish, Fundulus, which is not affected by the osmotic pressure of the solutions used in the experiments. In sodium chloride or potassium chloride solutions, of the concentration in which these salts exist in sea water, the fish only lived a few days; whereas in calcium or magnesium chlorides they lived indefinitely. But, in contrast to what we have seen to be the case in the heart, it was found that salts of sodium and potassium, present together in certain proportion, mutually deprived one another of toxic action. In the heart, as will be remembered, the presence of calcium is necessary in addition. A further demand is made by the sea water plant, *Ruppia maritima*, which requires, according to the investigations of Osterhout (1906), no less than four salts, viz., all the cations present in any quantity in sea water, namely, sodium, potassium, calcium, and magnesium.

Returning to the experiments of Loeb and Wasteneys, direct evidence is given that the effect is of one cation on another, and not of an opposite ion. The fact that above a certain concentration of potassium chloride, no neutralisation of its toxic effect by a sodium salt is possible, suggests a partition of some kind at the cell membrane and most probably an electrical adsorption. We have seen above (page 104) that there is no evidence for the formation of chemical compounds of protein with neutral salts, whether they be called "ion-proteids" or by other names. We may also call to mind that one substance may displace another from its state of adsorption, provided that in the process there is a further diminution of free energy of any kind.

If the proportion of sodium chloride to potassium chloride is much less than that in sea water, for example only eight molecules to one, the toxic action of the potassium is increased.

It is also interesting to note that calcium chloride itself is not toxic for Fundulus, so that, when it neutralises the toxic action of sodium and potassium chlorides on this fish, it is a case of a non-toxic ion neutralising a toxic one. Calcium has a much more powerful action in neutralising potassium than sodium has, about 500 times as great. Like that of sodium, however, the antagonism is limited and the interesting point about the fact is that the limit is the same, viz., no stronger solution than 6·6 c.c. of 0·5 molar KCl to 100 c.c. of water can be neutralised by any amount of either calcium or sodium salt nor by both together.

The fact that calcium has a much more powerful action than sodium has is not unexpected if we look upon the effect as exerted on the cell membrane. $Ca^{\cdot\cdot}$, as a bivalent ion, has much greater action on colloidal aggregation than sodium has. Strontium chloride has an effect about equal to that of calcium chloride; barium chloride has also a high value, but is very toxic. Magnesium chloride has relatively little action, so that the valency of the ion is not the only factor concerned.

Sodium chloride, in the concentration in which it exists in sea water, cannot be neutralised by potassium chloride alone, calcium must also be present.

A further interesting fact is that the toxic action of acids is also abolished by sodium ions and still better by calcium ions.

The opposite effects produced by sodium and calcium on the cell membrane have been referred to previously, and Clowes (1916) has shown that the reversal of phases in systems of oil and water is acted upon in an opposite way by these salts. It is difficult to understand why the effects of a univalent and a bivalent

cation should not only differ quantitatively, but be of opposite sign. Clowes suggests that the opposition is really one between cation and anion. In the case of calcium chloride, the cation is more powerfully adsorbed and reactive than the anion; in that of sodium chloride, the anion is adsorbed to a greater degree than the cation. Hence the possibility of making a balance between them and the reason why the necessary concentration of the calcium salt is so much less than that of the sodium salt. This view is confirmed by the fact that sodium citrate is more effective than the chloride in balancing calcium chloride, because the citric anion is more adsorbed than the chlorine ion.

According to Zwaardemaker (1918), equally radio-active amounts of radium, emanation, thorium, or uranium were equal in their capacity of replacing potassium, which is also slightly radio-active. Clark (unpublished) finds that uranium will not replace potassium in the sense that rubidium does.

If electrical phenomena play a part in the action of ions in general, it is possible that the affinity of an ion for its charge may have to be taken into account, as insisted upon by A. P. Matthews (1904). The most active ions would be expected to be those which part with their charges most easily. Although we must admit, with this author, that physiological action has frequently no connection with chemical structure, for example, beryllium sulphate, lead acetate, sugar, phloroglucinol and saccharin all taste sweet, it is undoubtedly going too far to say that all actions of enzymes or toxins have nothing to do with chemical structure, or that the action of a lead or other salt on the living organism is determined by the character and number of its electrical charges and by the ease with which it parts with these charges, and by nothing else.

ACTION OF SALTS IN PARTICULAR INSTANCES

In order to realise the many and various ways in which electrolytes intervene in physiological processes, it will be instructive to refer briefly to a few typical cases; some of these will require more detailed treatment in future chapters, so that they may be merely mentioned here.

The illustration by Hoeber (1911, p. 444) of our methods of regarding the combined effect of the various ways in which such actions may be exercised, is an apt one. He likens our conceptions to a mirror, which, in its present condition, does not give a sharp image. If the image appears to be a confused one, we must not jump to the conclusion that the mirror itself is an inappropriate one and distorts the object to be reflected, but that it is not sufficiently polished to show fine details as well as it does the coarser outlines. The physical chemistry of colloids, to mention one fact only, is still too full of gaps to answer all that it may be capable of.

It is perhaps well to name again the possible ways in which a salt or other electrolyte may act; the electrical charge, as such, has its effect; there is also the effect on the solvent, shown by lyotropic actions, and frequently expressed in the "Hofmeister series"; finally, we may have effects, not included in any of these and more nearly related to purely chemical action, so that they are often exerted by the salts of one element alone, or by those of closely related ones.

The Sign of the Electrical Charge on Cell Membranes, as worked out by Mines (1912), is the first of these general effects to which we may call attention.

On Adsorption by Surfaces.—When a substance with an electrical charge is adsorbed by the surface of a colloid, the amount adsorbed depends greatly on the sign of the charge of the surface, whether similar or opposite to that of the substance adsorbed. By electrolytes, the charge of the surface can be annulled or reversed.

Hæmoglobin.—An important action of electrolytes on the dissociation of oxyhæmoglobin, described by Barcroft and Camis (1909), probably depends on the colloidal nature of this substance. At a given pressure of oxygen, less of this gas is taken up by hæmoglobin in presence of salts than in pure water. For example, at 30 mm. of mercury of oxygen pressure, the percentage saturation in water is 85, and in Ringer's solution only 60. The effect is still more marked with acids, and is a delicate indication of the hydrogen ion concentration in blood. The importance of these facts will be seen later in connection with the supply of oxygen to the tissues.

Enzyme Action.—Many enzymes are inactive in the absence of electrolytes. In some cases, this appears to be due to the facilitation, by salts, of adsorption between enzymes and their respective substrates.

Hæmolysin.—It was shown by Gengou (1908) that the hæmolysin of the serum of the eel is inactive without electrolytes.

Secretion.—It will be seen later that the excitatory action of extracts of the duodenal mucous membrane in causing the pancreas to secrete is not shown in the absence of electrolytes.

Electrical Excitation.—Since salts are always present in living tissues, it is clear that the result of applying an electrical current must be the separation to a greater or less extent of the ions of opposite sign at the two electrodes. The exciting effect of the cathode and the inhibitory effect of the anode is, no doubt, connected with this fact. The opposite action of $H^{\cdot}$ and OH' ions is a familiar fact and has been already referred to.

Smooth Muscle.—Hooker (1911) shows in experiments on perfusion of the blood vessels of the frog with saline solutions, that calcium produces contraction of the muscular coat, while potassium and sodium cause relaxation. Gaskell (1880-82, pp. 55 and 56) had already shown that acids cause relaxation, and alkalies cause contraction.

Pigment Cells.—The fish, Fundulus, contains in its skin yellow and black pigment cells. It has been shown by Spaeth (1913) that potassium salts expand the former, contract the latter. Sodium salts have an opposite effect on both. By photographing the same cell, it is seen that the expansion and contraction does not concern the cell as a whole, since the processes remain permanently of the same form. The pigment granules migrate inside the processes to and from the centre of the cell.

We may pass on to some special actions of individual ions.

Calcium.—Although, in certain processes, calcium can be replaced by other alkaline earths, there are others in which this is not so. Barium, for example, is especially toxic to the animal organism. The property of calcium to favour consolidation or stability in colloidal systems, in opposition to that of the alkali metals, which tend towards liquefaction in some cases, is, no doubt, rightly indicated by Hoeber (1911, p. 446) as being of great importance in the explanation of the physiological action of calcium. Moreover, the same author points out that the action of a bivalent ion is much less violent than that of a multivalent ion and is much more easily reversible.

We have already seen the necessity of calcium for the heart beat of the vertebrate, and Lovatt Evans (1912, 2, p. 410) has shown that the same statement applies to that of the snail. The latter, however, is able to stand a much higher concentration than that of the frog, beating quite normally in 2 per cent. calcium chloride. Barium is quite as toxic as to the vertebrate heart, one part in 20,000 causing a marked systolic condition.

Locke showed (1894) that calcium is also necessary for the transference of the excitatory process from nerve to muscle and Overton (1904) showed that it is equally necessary for the transmission of the excitatory state through the synapse of a nerve fibre with a nerve cell. According to Busquet and Pachon (1908) when the action of the vagus nerve on the frog's heart has been stopped by perfusion with pure sodium chloride solution, as shown by Howell (1906), addition of calcium chloride in extremely small amount is sufficient to restore the inhibitory action to the vagus nerve.

Clark (1912, p. 12) has shown that digitoxin (the active substance of the foxglove) is inactive without calcium.

In Fig. 66 the heart of the frog is seen to be at first beating normally in Ringer's solution. At A, calcium-free Ringer's solution is perfused to wash away calcium, and at A', repeated circulation of the same 3 c.c. of the same solution is established. The feeble beat seen in the tracing continues for hours under these conditions. At B, a trace of calcium chloride is added; the beat returns to normal. At B', 0·01 mg. of digitoxin is added and at C, perfusion with calcium-free Ringer's solution is recommenced. It will be seen that, although the beat is somewhat stronger and slower, the typical systolic tone, which the drug normally produces, is absent. At D, the normal amount of calcium chloride (0·02 per cent.) is

added ; the systolic arrest appears immediately. It is to be noted that the amount of calcium added is only just about the amount required to neutralise the potassium present in the solution, so that the effect is not due to the calcium but to the digitoxin, which had been in abeyance until the calcium was added.

Hamburger (1910) finds that calcium increases the amœboid movement of phagocytic leucocytes, while barium, strontium and magnesium have no such effect.

Chiari and Januschke (1910) describe a remarkable action of calcium. If sodium iodide is injected intravenously into dogs, fluid is exuded into the pleura and pericardium, and œdema of the lungs is produced. But, if calcium chloride is injected simultaneously, these cavities remain quite dry. Exudation produced in other ways is also subject to the same effect of calcium. Inflammation and swelling of the eyelids, caused by oil of mustard, is prevented by previous subcutaneous injection of calcium chloride. Recent investigations, however, have shown these results to be very doubtful.

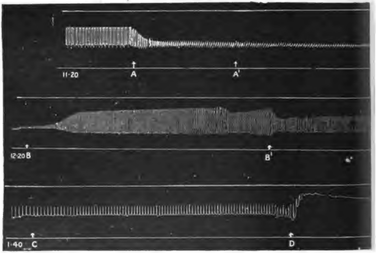

FIG. 66. INACTIVITY OF DIGITOXIN ON THE FROG'S HEART IN ABSENCE OF CALCIUM.

(Clark, 1912, fig. 7 ; *Proc. R. Soc. Med.*)

An interesting fact noted by Mines is that *strontium* can be *antagonised by calcium* in respect to its action on the heart muscle. The characteristic tonic effect of calcium is possessed to a greater degree by strontium ; but, if to a Ringer's solution, containing sufficient strontium to give a marked prolongation of the beat, there be added the normal amount of calcium salt, the beat returns to its correct form. It is difficult to say in what way this effect is produced ; possibly calcium lowers some form of surface energy to a greater extent than strontium does, and therefore displaces it from the cell membrane, or calcium may give up its electrical charge with greater ease than strontium does, and thus maintain the proper colloidal consistency of the membrane.

In the absence of calcium, the heart of the frog is unable to give contractions. The continuance of the electrical change shows that the excitatory process goes on (see Mines, 1913, 3, p. 231), and Locke and Rosenheim (1907) found that glucose is still consumed. It appears that there is some break in the mechanism of conversion of chemical energy to contractile stress. The state of the active surfaces (see below, page 448), in the absence of calcium, is such that the

increase of surface energy, normally produced by the liberation of lactic acid, cannot take place.

If the blood vessels of the frog are perfused with Ringer's solution and a trace of adrenaline added, a marked constriction is shown by a slowing of the rate of flow. According to R. G. Pearce (with Asher, 1913, p. 274), if pure isotonic sodium chloride is used, adrenaline causes dilatation of the vessels. It appears that calcium is necessary for the normal effect of adrenaline on the sympathetic nerve-endings. In experiments of this kind caution is necessary on account of the spontaneous rhythmical changes which are apt to occur in the frog's blood vessels under saline perfusion, as I have described (1901, 1). In fact, I have been unable to confirm Pearce's results. This apparent reversal of an excitation to an inhibition will come up for discussion again in a later page.

There are two particular phenomena of physiological interest which appear to be colloidal aggregations. These are the coagulation of the blood and that of milk by rennet. For both, the presence of calcium is required. In the former case the fact was first definitely proved by Arthus et Pagés (1890), although the favouring action of calcium salts had been noticed previously and it had been shown by Ringer and Sainsbury (1890) that barium and strontium had the same effect, but in less degree. Arthus et Pagés, also, showed that strontium could replace calcium.

In the clotting of caseinogen in milk by rennet, it is first converted into a form which is precipitated by calcium (Ringer, 1890). Fraser Harris (1896) showed that strontium and barium can replace calcium.

Magnesium.—Meltzer and Auer (1905) describe how the subcutaneous injection into a rabbit of 1·7 g. of magnesium sulphate per kilogram produces in thirty to forty minutes deep anæsthesia and paralysis and (1908) how this effect is removed in a few seconds by the intravenous injection of about 8 c.c. of 3 per cent. calcium chloride. The same experimenters (1909) show that the application of a molar solution of magnesium sulphate to the surface of the medulla oblongata causes, within fifteen minutes, abolition of the functions of all the medullary centres. Meltzer (1913) points out the value of a preliminary dose of magnesium sulphate in ether narcosis. If 0·6 g. of crystallised magnesium sulphate per kilogram of animal is given intramuscularly to rabbits (or 0·8 g. to dogs) a very small effect is produced; but if ether be given, profound anaesthesia results from one-tenth of the dose usually required for mild anaesthesia.

Sodium.—It was shown by Overton (1904) that frog's muscle immersed in isotonic cane-sugar (7 per cent.) loses its excitability, and that restoration can be brought about by a sodium salt or, in a less degree, by a lithium salt, but not by salts of potassium or ammonium.

Nerves behave in a similar way.

Potassium.—The action of potassium is, in the main, but not always, a paralysing one, as seen in the case of the heart. At the same time, its presence is necessary to control the opposite action of calcium.

It is probable that the powerful physiological action of potassium may be connected with the rapid rate of migration of its ions. If the table on page 177 be consulted, it will be seen that these ions have a higher transport number than any other cation, with the exception of hydrogen. This fact will enable them to play a prominent part in the phenomena connected with the electric charge on surfaces. In the formation of a Helmholtz double layer, potassium ions will outdistance other cations and, therefore, tend to be in excess in the positively charged side of the layer. See also the remarks on page 214 on the radio-activity of this metal.

Howell (1906) showed that, in the absence of potassium salts, the vagus nerve loses its power of inhibiting the beats of the heart, and the similarity between the action of potassium and that of the vagus nerve suggested to him (1906, p. 291) the hypothesis that the action of this nerve might depend on the setting free, in some way, of potassium. Howell and Duke (1908) found that an increase of potassium could be detected in a small amount of Ringer-Locke's solution which had passed repeatedly through a mammalian heart under vagus inhibition.

Hemmeter, however, (1913) was unable to find any difference in the potassium content of the ash of normal and inhibited hearts, but this would scarcely be expected to be the case. In the blood contained within the heart of the dog-fish, under both conditions, again no difference was found, but the amount diffusing into the blood might easily be within the limits of the experimental error of the method used, that of ordinary chemical analysis. Of more interest

is the fact that the blood passing by crossed circulation from the heart of one dog-fish to that of another had no effect on the latter when the former was inhibited by the vagus nerve. But, in experiments of this kind, negative results are less convincing than positive ones.

Chlorine.—Turning our attention to anions, perhaps the most striking action is that of chlorine on the central nervous system, according to the work of von Wyss (1906). When sodium bromide is given in large doses, the chlorine content of the blood can be reduced to one-third. The exact cause of this is disputed, but the interesting point is that, at this stage, characteristic symptoms of paralysis set in. According to von Wyss, these symptoms are not due to accumulation of bromine, but to loss of chlorine, since they are rapidly cured by giving sodium chloride. Moreover, while ammonium chloride is effective, sodium nitrate or sulphate or magnesium sulphate is without action. Grünwald (1909) obtained similar results by depriving rabbits of chlorine in their food and administration of diuretics.

Carbon Dioxide.—Whether carbon dioxide or CO_3'' ions have any special action on cell processes apart from that of the hydrogen ion also present in solutions of carbon dioxide, is doubtful. It is held by some, for example, Laqueur and Verzár (1912), that carbon dioxide as such has an exciting effect on the respiratory centre, but the experiments are not convincing (see Chapter XXI.). Rona (1912) stated that it has a similar one on the movements of the intestine. The addition of sodium bicarbonate to a saline solution containing neither bicarbonate nor phosphate, caused the movements of an excised intestine to change from an irregular character to a perfectly regular one. This was apparently not due to diminution in hydrogen ion concentration, since the addition of bicarbonate had the same effect if its solution were previously brought to the same hydrogen ion concentration as the solution to which it was added. Also the production, by sodium hydroxide, of the same degree of alkalinity as that caused by the bicarbonate, with glycine as "buffer," had no effect.

Jacobs (1920) confirms the greater effect of solutions containing carbon dioxide than those of other acids of an equal $H^{\cdot}$ ion concentration. The interesting suggestion is made that, since the cell membrane must be permeable to carbon dioxide in solution, although not to the $H^{\cdot}$ ion, the former is allowed to enter the cell as CO_2 and, becoming electrolytically dissociated, after reaction with water, $H^{\cdot}$ ions are produced in effective contact with cell structures, whereas acids in general do not enter the cell until they have damaged the cell membrane.

SALTS OF WEAK ACIDS WITH WEAK BASES

When a strong acid is added to a strong base in dilute solution, there is a considerable fall in the electrical conductivity of the mixture as compared with the sum of those of the two reagents separately. Since the salt formed is dissociated to as great a degree as the acid or base, the diminution must be due to the disappearance of the fast moving ions $H^{\cdot}$ and OH'.

For example, the conductivity of a 0·05 molar hydrochloric acid at 21·8° is 17,945 reciprocal megohms; that of a similar concentration of sodium hydroxide is 9,695 reciprocal megohms; together 27,640, whereas 0·05 molar sodium chloride is only 4,995. In the solution of the salt there are, per 20 litres, 1 molecule of Cl' ions and 1 molecule of $Na^{\cdot}$ ions, together 2 molecules, very nearly; in 20 litres of hydrochloric acid, 1 molecule of $H^{\cdot}$ ions and 1 molecule of Cl' ions; in 20 litres of sodium hydroxide, 1 molecule $Na^{\cdot}$ ions and 1 molecule OH' ions; so that, if uncombined when mixed, there would be in all 4 molecules. But, even if we double the value of the conductivity of the sodium chloride solution to allow for this, we only have 9,990, instead of 27,640. It is evident that the diminution is only partially due to the disappearance of $H^{\cdot}$ and OH' ions in combination as water, but that the slower rate of migration of the $Na^{\cdot}$ and Cl' ions also plays a part.

Again, if we neutralise a weak base, such as aniline, with a strong acid, we get a diminution of conductivity, or if a weak acid is neutralised with a strong base.

On the other hand, if we take a weak base and a weak acid, the conductivity of the salt is higher than the sum of those of the base and acid together. This is because the salt is more highly dissociated, electrolytically, than either the base or the acid, so that there is an actual increase in the number of ions present.

It is not easy to see why, to take a specific case, the compound of the acetic anion with hydrogen ion is very little dissociated, whereas when it is combined with the cation of aniline there is considerable dissociation.

The fact is probably of some importance in physiological processes. The organic acids and bases produced in cell metabolism are for the most part of the weak class, that is, very little electrolytically dissociated; when they combine, the salt is highly dissociated, so that a number of ions make their appearance. So far, then, as the properties of a substance are those of its ions, the salts of weak acids with weak bases are more powerful agents than the substances from which they are formed.

There are two practical points of interest in connection with this question.

In the first place, the fact gives us a very convenient means of following the course of a tryptic digestion. The weak amino-acids produced, when they combine with the ammonia used to give the requisite degree of alkalinity, or with diamino-acids, acting as bases, give rise to a considerable increase in conductivity.

The conductivity of leucine in 0·05 molar strength at 22° is only about 3 reciprocal megohms, that of ammonium hydroxide in the same conditions is 232 reciprocal megohms, together 235. When mixed, the salt formed is fairly highly dissociated and the solution has a conductivity of 1,548 reciprocal megohms. This may be compared with aniline acetate; aniline in 0·05 molar solution has a value of 13, acetic acid in the same concentration is 330, together 343; while aniline acetate, 0·05 molar, is 1,518.

In the second place, we obtain some information as to the relative strengths of an acid and a base. An acid which is weak towards a strong base may be relatively strong towards a weaker base.

For example, salicylic acid, which has a dissociation constant of 102×10^{-5}, when combined with ammonium hydroxide, gives an *increase* of conductivity, that is, it is a weak acid towards the base, ammonium hydroxide; when combined with aniline, on the other hand, there is a *fall* in conductivity, that is, it is a relatively strong acid towards the very weak base, aniline. Maleic acid (dissociation constant $= 1170 \times 10^{-5}$) is a strong acid to both bases and acetic acid (dissociation constant $= 1{\cdot}8 \times 10^{-5}$) is weak to both bases. The mono amino-monocarboxylic acids are too weak as bases to combine with acids as weak as acetic acid. On the other hand the diamino-mono-carboxylic acids are sufficiently strong as bases to combine with acids as strong as the mono-amino-dicarboxylic acids. For example, I found that diamino-propionic acid, 0·17 molar, had, at 40°, a conductivity of 1,672 reciprocal megohms, glutamic acid, 0·095 molar, had a conductivity of 950 on the same scale, together 2,622; a solution containing both in the same concentration as before had a conductivity of 5,142 reciprocal megohms, showing that combination had taken place (Bayliss, 1909, 2).

It is to be noted that the use of the words "weak" and "strong" in the above connection is to be taken only as referring to their relative power of combining with weak acids or bases respectively. It does not conflict with the expression as used in reference to the electrolytic dissociation of their solutions, which is an absolute measurement of their strength as compared with one another.

AMPHOTERIC ELECTROLYTES

There is an important class of substances, already referred to incidentally in connection with the colloidal properties of proteins, which can act either as acids or bases; that is, they dissociate with the formation of H· and OH′ ions. We have seen that water is a member of this class and we have now to turn our attention to a very important series of substances, the amino-acids. These owe their nature as both bases and acids to the fact that they contain one or more NH_2 groups, together with one or more COOH groups.

Amino-acetic acid or glycine may exist in the hydrolysed state in water as :—

$$\begin{array}{l} CH_2{-}NH_3OH \\ | \\ COOH. \end{array}$$

For convenience, we may call the radical which is combined with H and OH, R, which is :—

$$\begin{array}{l} CH_2{-}NH_3{-} \\ | \\ COO{-} \end{array}$$

in the case of glycine. Then, according to the investigations of Bredig (1899) and of J. Walker (1904), the solution contains the following molecules and ions :—

H·, OH′, HR·, ROH′, HROH, and R.

Whether R is to be looked upon as an ion with both a negative and a positive charge is doubtful. If so, it is formed by giving off both H· and OH′ ions and would be represented thus:—

$$\begin{array}{l} CH_2NH_3^{\cdot} \\ | \\ COO' \end{array}$$

in the case of glycine and is sometimes known as a "hermaphrodite" ion. In Bredig's scheme, however, it is represented as devoid of charges and is probably, in fact, an internal anhydride:—

$$\begin{array}{l} CH_2NH_3 \\ | \quad / \\ COO. \end{array}$$

As such, we must suppose that the two groups combined have opposite charges, so that it is not impossible that they might exist as such on a single ion. An interesting suggestion is made by Bredig (1894, p. 323), as to the length of the chains which can exist without self-neutralisation. If a sufficiently long chain could be formed, having opposite charges at the ends, it should be possible by optical means to detect an orientation to an electrical current passed through the solution. Bredig, himself, was unable to detect any sign of this in the case of betaine.

It is unnecessary to remark that an ion with two opposite charges moves to neither electrode, being equally attracted to both, so that it can take no part in the conduction of a current. In this aspect, it is not, in any case, entitled to the name of an ion, in Faraday's sense.

As we have seen above (page 105), there is no evidence that an amino-acid can combine with the positive and negative ions of a neutral salt simultaneously. A "hermaphrodite" ion should be able to do this.

The various ions enumerated above as present in solutions of the amino-acids exist in very small concentrations, so that their electrical conductivity is very low, especially in the case of the mono-amino-monocarboxylic series. The acidic and basic groups are mutually antagonistic, so that both dissociation constants are very small. The mono-amino-monocarboxylic acids are very weak indeed, both as acids and as bases. The carboxyl group is a little stronger as acid than the NH_2 group is as base, so that the acid properties very slightly preponderate.

When we have another COOH or another NH_2 group added on, as in aspartic acid or lysine respectively, the acidic function is considerably increased in the first case and the basic function in the second.

From Winkelblech's investigations (1901) it is interesting to note that, when the strength of the acid group considerably exceeds that of the basic one, as in taurine (amino-ethyl-sulphonic acid), salts are formed only with bases, not even with acids as strong as hydrochloric acid. Conversely, if the basic group is considerably stronger than the acid one, as in betaine (tri-methyl-glycine), then salts are formed only with acids. It is also somewhat unexpected to find that, comparing glycine, alanine, leucine, sarcosine and betaine, the stronger acid is at the same time the stronger base, but the fact appears to hold only for the mono-amino-mono-carboxylic series.

As to the methods of determining the two dissociation constants, one of these is that of conductivity measurements of their salts with hydrochloric acid and with sodium hydroxide, and another is that of hydrolytic dissociation. The papers by Lundén (1908) and by Winkelblech (1901) may be consulted.

I insert here the values of the dissociation constants of a few amphoteric electrolytes, at 25°, taken from Lundén's work (1908, p. 81).

	Acidic.	Basic.
Theobromine	$1{\cdot}1 \times 10^{-10}$	$4{\cdot}8 \times 10^{-14}$ (at 40°)
Xanthine	$1{\cdot}2 \times 10^{-10}$ (at 40°)	$4{\cdot}8 \times 10^{-14}$ (at 40°)
Leucine	$1{\cdot}8 \times 10^{-10}$	$2{\cdot}3 \times 10^{-12}$
Glycine	$1{\cdot}8 \times 10^{-10}$	$2{\cdot}7 \times 10^{-12}$
α-Alanine	$1{\cdot}9 \times 10^{-10}$	$5{\cdot}1 \times 10^{-12}$
Arsenious acid	$6{\cdot}0 \times 10^{-10}$	$1{\cdot}0 \times 10^{-14}$
β-i-Asparagine	$1{\cdot}35 \times 10^{-9}$	$1{\cdot}53 \times 10^{-12}$
Histidine	$2{\cdot}2 \times 10^{-9}$	$5{\cdot}7 \times 10^{-9}$
Tyrosine	$4{\cdot}0 \times 10^{-9}$	$2{\cdot}6 \times 10^{-12}$
Glycyl-glycine	$1{\cdot}8 \times 10^{-8}$	$2{\cdot}0 \times 10^{-11}$
Aspartic acid	$1{\cdot}5 \times 10^{-4}$	$1{\cdot}2 \times 10^{-12}$

With regard to proteins, we have seen in dealing with them from the colloidal point of view how the effect of acid and alkali on the sign of their electrical charges is explained by their nature as amphoteric electrolytes. A further proof of this fact is afforded by the measurements of the freezing points of their salts with acid and alkali, as obtained by Bugarszky and Liebermann (1898, p. 72). In the table below, the first column gives the number of grams of egg albumin added to 100 c.c. of the acid, base, or salt in 0·05 molar concentration, and the three remaining columns give the depressions of the freezing point in each of these cases.

Grams.	△ for HCl.	△ for NaOH.	△ for NaCl.
0	0·186	0·181	0·183
0·8	0·172	0·162	0·191
1·6	0·146	0·151	0·194
3·2	0·101	0·116	0·199
6·4	0·087	0·097	0·203

It will be seen that there is a considerable diminution of △ in the cases of acid and base, due to formation of salts with the protein. In the case of the neutral salt there is no such effect. The contrary effect, a rise of △ with the sodium chloride, is, in fact, due to the albumin itself, since 6·4 g. of the protein in 100 c.c. of water gave a freezing point depression of 0·022; this, added to 0·183, gives 0·205, as in the table, within the limits of experimental error.

ACTION OF ELECTROLYTES IN EXTREME DILUTIONS

The powerful effect of *hydrogen and hydroxyl ions* in traces has been exemplified in the case of the heart. Further instances will occur in the course of this book.

One or two striking cases of the action of inorganic salts in minute quantities may be referred to here.

Elissafoff (1912) showed that the effect of the quadrivalent *thorium* ion on the surface charge of quartz was such as to lower it by 50 per cent., when the solution contained only one gram-ion in a thousand million grams of water.

The extraordinary effect of *zinc* in traces on the growth of moulds was discovered by Raulin (1870, 1 and 2), as also that of manganese. This observer was doubtful whether the effect of manganese salts was not due to traces of zinc, and the matter was further worked out by Bertrand and Javillier (1912). They found that manganese itself actually has an effect of this kind. One part of manganese in one million of the culture solution raises the crop of Aspergillus from 0·610 to 0·631 and the effect continues to increase even up to one part in 100. In further work it was found that the combination of zinc with manganese was more effective than either alone. To take an example:—

	Weight of Crop.
Control - - - - -	1·45
With Zn, 1 : 500,000 - - -	4·10
With Mn, 1 : 5,000 - - - -	2·79
With both together - - -	4·35

The data also show the really astonishing effect of zinc alone. In another experiment, indeed, we find that the addition of one part of zinc to twenty-five millions of solution increases the crop from 3·00 to 4·54, that is, by more than 50 per cent., and one part in ten millions nearly doubles it.

The authors point out how important is this function of elements present only in traces; they regard it as being of a catalytic nature. We shall have occasion later to return to the question of the effect of substances, not only inorganic ones, which, although present only in infinitesimal amount, are, as it seems, absolutely indispensable to the normal functional capacity of protoplasm.

From the work of Raulin it appeared also that iron in traces had a great effect on the normal production of the fructification (conidia) of the mould. Bertrand (1912), having been able to prepare solutions in a great state of purity, found that, although iron and zinc might both be present, there were no conidia formed unless manganese was also present. If any one of these three elements is wanting, or present in too small a quantity, complete normal growth is impossible. But whereas vigorous growth of mycelium takes place with iron and zinc alone, no conidia are formed in the absence of manganese.

OLIGODYNAMIC ACTION

The opposite phenomenon to the favourable action by traces of zinc on Aspergillus are to be found in the toxic action of certain metals, especially copper, more particularly to the higher organisms.

In a posthumous paper by Nägeli (1893), some very important results are described in relation to this question. It was noticed that ordinary distilled water was rapidly fatal to Spirogyra, just as Ringer and Phear at a later date (1895) found that it was to tadpoles.

Nägeli discovered that the toxic action was due to the presence of minute traces of compounds of various heavy metals in the water. Tap water, which originally did not show this property, became poisonous after being in contact with metallic copper, mercury, lead, tin, iron, or silver. It was also found that the addition of various insoluble solids, such as paper, wool, paraffin, or of certain colloids, such as gum or gelatine, deprived the water so treated of its toxic character. From what has been said in previous chapters of this book, when dealing with the colloidal state and the phenomena of adsorption, the explanation of this neutralising power of surfaces will be obvious. The toxic metal is present either as hydroxide or carbonate in the colloidal state; this, as an electro-positive colloid, will be strongly adsorbed by electro-negative surfaces, such as those used by Nägeli. The fact noticed by Ringer (1886, p. 292) that calcium phosphate is more effective in neutralising the toxic properties of distilled water than calcium chloride is, is easily explained by the greater precipitating action of the trivalent PO_4''' ion on an electro-positive colloid than that of the univalent Cl' ion.

Nägeli estimated the amount of copper present in 12 litres of distilled water, which had been for four days in contact with 12 two-pfennig pieces. It contained one part in seventy-seven millions. This water was powerfully toxic to Spirogyra, killing it in one minute. On account of the very small quantity of copper in the water, Nägeli gave the name of "*oligodynamic*" to the action in question.

Locke (1895), in repeating these experiments, found that, of the various metals tested, copper was by far the most toxic. A strip of bright copper, 4·5 by 1·5 cm. in dimensions, placed for twenty hours in 200 c.c. of distilled water, made the water toxic to tadpoles and to the river worm, Tubifex. Brass had the same effect as copper, but zinc, although toxic, was not so powerfully active, while tin appeared to be innocuous.

Raulin, in the course of the work referred to in the preceding section, had also noticed that one part of silver nitrate in 1,600,000 of the culture medium was sufficient to prevent germination of the spores of Aspergillus; in fact, if the medium is contained in a silver vessel, sufficient metal is dissolved to prevent growth therein.

Ringer and Phear did not attribute the toxic action of their distilled water to "oligodynamic action," but Locke, in the paper quoted above, showed clearly that the explanation lay in this fact, since distilled water condensed in glass had no injurious action.

It has been found that certain bright metals pass readily into the colloidal state when placed in contact with pure distilled water (see Traube-Mengarini and Scala, 1912). Thus lead, zinc, iron, tin, aluminium, copper, and nickel form, in this way, colloidal solutions in which the dispersed phase is, at first, in the metallic state, but subsequently becomes hydroxide.

SUMMARY

There is a group of substances, which, investigated in various methods, are found to show, in solution in water, a higher osmotic pressure than that corresponding to their molar concentration. All these substances are found to be conductors of electrical currents, that is, they are electrolytes, to use the name introduced by Faraday.

It is clear, therefore, that the molecules of electrolytes are split up, dissociated, in solution in water, so that there are more osmotically-active elements in their solutions than in those of non-electrolytes in the same molar concentration.

Since electrolytes conduct electricity by means of their "ions," which appear at the two electrodes (Faraday), the view was put forward by Arrhenius that these ions exist in solutions of electrolytes in ordinary conditions, independently of the passage of electrical currents.

Evidence of various kinds has been brought to show that this is the case. Hydrochloric acid, for example, is more or less completely split up into hydrogen ions, each carrying a unit positive charge, and chlorine ions, each carrying a unit negative charge. This is known as "electrolytic dissociation."

The more dilute the solution, the more complete is the dissociation.

The power of conducting a current depends both on the actual number of ions engaged in the carriage of the charges and also on the rate at which they move. The rate has considerably different values for different ions and is in relation not only to the atomic or molecular weight of the ion, but to the number of molecules of water which are attached to it (Hydration of Ions). The value is constant for each ion under similar conditions. The absolute rate of movement is slow. Hydrogen ions, the most rapid, have a velocity, under a potential fall of one volt per centimetre, of only 0·0033 cm. per second; but the rate is, of course, dependent on the force producing the motion.

The reason why it is impossible to separate the oppositely charged ions by diffusion, or other means except an electrical one, is the enormous electrostatic attraction between them, which prevents a positive ion from being separated from its fellow negative one beyond infinitesimal distances.

When, however, one of the ions moves faster than the oppositely charged one, it does actually form a layer in front of that of the more slowly moving ions, at a very minute distance. This phenomenon is known as the "Helmholtz double-layer" and is the cause of the appearance of an electromotive force at the boundary surface between solutions containing ions of differing mobility.

The source of the energy required to dissociate the molecules of electrolytes when dissolved in water is discussed in the text, as also the relation of the process to the dielectric constant of the solvent.

While the equilibrium between non-dissociated molecules and ions in the cases of weak acids and bases obeys the law of mass action, as shown by their behaviour on dilution (Ostwald's Dilution Law), that of strong acids, strong bases and salts obeys a different law. The explanation of this fact has not yet been given. It has been suggested by Noyes and his co-workers that there may be two different kinds of combination between ions to form molecules, one rather of an electrical nature and somewhat loose, the other more strictly chemical and more stable. The former would be the case with the strong electrolytes.

In their intervention in physiological processes, electrolytes may be said to act mainly in three ways. By the electrical charges on their ions, as in colloidal phenomena; by their effect on the properties of the solvent, "lyotropic" action; and by the purely chemical properties of their ions or molecules.

The important part played by acidity and alkalinity shows the value of the electrolytic dissociation theory in an especially striking way. These properties of solutions can be expressed by the numerical values of their concentration in

hydrogen or hydroxyl ions. A weak acid or a low degree of acidity is such because there are relatively few hydrogen ions present.

The different degrees of dissociation enable us to express the strength of acids or bases, with the exception of those which do not obey the law of mass action, in numerical quantities, known as their "dissociation constants" or "affinity constants."

To understand the meaning of these, a brief account of the law of mass action is introduced. This law states that the rate of any reaction is proportional to the masses of the reacting substances. The meaning of "velocity constant" and of "equilibrium constant," as the ratio of the two velocity constants of the two opposite reactions in a reversible system, is explained.

The "dissociation constant" is the equilibrium constant of the reversible reaction of electrolytic dissociation. Since it presupposes that the law of mass action is followed, it can only be given in the case of weak electrolytes.

Instances of the activity of hydrogen and hydroxyl ions in cell processes are given; such are the action of enzymes, the character of the heart beat, and so on.

Hence accurate methods of determining the hydrogen ion concentration are indispensable. The methods of the use of indicators, the gas electrode and the hydrolysis of esters or cane-sugar are described.

In connection with the hydrogen electrode, the theory of electrode potentials is discussed and the precautions necessary in the use of the method with blood are pointed out.

In the use of the method of hydrolysis of esters, etc., the peculiar effect of neutral salts in increasing the hydrolytic action of a given concentration of strong acid has to be taken into account.

The powerful effect of changes in hydrogen ion concentration on physiological processes requires the existence of mechanisms for the prevention of any considerable changes of this kind.

There are two chief chemical systems in which the reactions occurring on the addition of acid or alkali are of such a nature as to require the addition of comparatively large amounts of acid or alkali in order to produce any marked change in the hydrogen ion concentration. These systems are the bicarbonate-carbon-dioxide system and that of the acid and alkaline phosphates. The former is the more widely occurring one, although the phosphate system is also of importance in protoplasmic reactions. Proteins appear only to play a part when the $H^{\cdot}$ ion concentration is higher than 10^{-5} or lower than 10^{-10}.

In the reactions referred to in the previous paragraph, the phenomena known as "hydrolytic dissociation" play an important part. This process is shown to occur by the presence of free acid and free base in solutions in water of salts of weak acids or bases. It is due to two facts; the first is that water itself is a very weak electrolyte, being to a minute extent electrolytically dissociated into hydrogen and hydroxyl ions; the second is the slight electrolytic dissociation of weak acids and weak bases. By interaction of the four ions thus present, there is an excess of hydroxyl ions when the base is the stronger and of hydrogen ions when the acid is the stronger. A very small degree of hydrolysis of the salts of many organic acids with strong bases is frequently to be met with, even in cases where the acid would be expected to be a weak one.

In the bicarbonate system, the escape of carbon dioxide as gas, when the hydrogen ion concentration of the system rises, is an important factor in the maintenance of neutrality. Numerical results are given in the text, showing the efficiency of the system at hydrogen ion concentrations not very far above or below that of neutrality.

Carbon dioxide possesses powers of neutralising alkali of a degree not shared

by any other acid, except hydrogen sulphide, a fact which is significant in view of its universal production as the result of oxidations in the organism.

The effect of rise of temperature on the bicarbonate system is to increase the alkalinity, on account of the greater temperature coefficient of electrolytic dissociation of water than of sodium bicarbonate.

The hydrogen ion concentration of blood at 38° is $0{\cdot}4 \times 10^{-7}$ and the hydroxyl ion concentration is $7{\cdot}2 \times 10^{-7}$ molar; that is, it is just on the alkaline side of neutrality. This concentration of hydrogen ions reacts alkaline to methyl orange or litmus, acid to phenolphthalein; the colour of neutral red in such a solution is orange.

The method of preparing solutions of known concentrations in hydrogen ions by the use of phosphate mixtures is described in the text.

The experiments of Ringer on the heart of the frog have shown that, for an efficient artificial saline solution to replace blood, it is not sufficient to take sodium chloride alone in isotonic concentration, but that the presence of potassium and calcium salts is indispensable in addition. In this action, it is the cation that is the necessary part of the salt.

There is evidence that the salt composition of the blood plasma of higher vertebrates is a relic of the composition of the ocean in pre-Cambrian ages. At this period, the blood plasma had the same salt content as the sea water, and when the ancestors of the present land vertebrates left the ocean at the close of the Cambrian epoch, they carried with them an adaptation to this particular concentration of salts.

The necessity of salts having "antagonistic" action towards each other's toxic properties applies to protoplasmic action in general.

A number of examples is given showing the intervention of electrolytes in physiological processes; enzymes, hæmoglobin, hæmolysin, secretion, muscular contraction, pigment cells, coagulation of the blood, transmission of excitation from nerve to muscle and from nerve fibre to nerve cell, action of drugs, phagocytosis, narcosis, the respiratory centre, are referred to briefly.

The salts of weak acids with weak bases have an importance in that they are much more strongly dissociated electrolytically than either the free acids or the free bases themselves.

Amphoteric electrolytes, of which proteins and amino-acids, next to water itself, are the most important, are capable of forming salts with either acids or bases, provided that these are fairly strong. There is no adequate evidence of combination with neutral salts.

There are certain heavy metals which have a very powerful action on living cells, even when in extremely minute concentration. Zinc and manganese greatly favour the normal growth of Aspergillus, while copper, lead, and some other metals have an intensely toxic action on the protoplasm of Spirogyra and animal cells. This latter effect is known as "oligodynamic" action.

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Indicators.

Prideaux (1919).

CHAPTER VIII

WATER, ITS PROPERTIES AND FUNCTIONS

THERE is no doubt that if water were as uncommon a liquid as, say, amyl-alcohol or toluene, it would be looked upon as endowed with the most wonderful properties. Common as it is, ancient philosophers like Thales regarded it as the origin of all things, and the development of science has shown how important it is in all the phenomena with which we have to deal. It is chosen to fix standards of density, of heat capacity and so on; most of the reactions with which chemistry is concerned take place in aqueous solutions. The action of water, in its several forms of ice, liquid or vapour, is the chief factor in geological changes. Finally, all physiological actions have their seat in systems containing water as an essential component.

We have already had occasion to take some account of its intervention in protoplasmic activity, in the production of the colloidal state, in permeability and osmotic pressure, and, in the previous chapter, in the dissociation of electrolytes. We turn now to consider its various physical and chemical properties in turn, together with their importance in vital processes. For many points to which attention is directed, I may acknowledge my indebtedness to the third chapter of L. J. Henderson's "Fitness of the Environment" (1913), to which the reader is referred for more details.

HEAT CAPACITY

Of all solids and liquids under ordinary conditions of temperature and pressure, water has the highest heat capacity, or specific heat. In other words, it takes more heat to raise the temperature of a given mass of water by a given amount, than it does in the case of any other of these substances. Liquid water is therefore chosen as the unit of specific heat, and in consequence also to define the unit of quantity of heat. The small calorie is that amount of heat required to raise the temperature of one gram of water from 0° to 1° C.

The law of Dulong and Petit, that the specific heat of an element varies inversely as its atomic weight, shows that a substance to have a high heat capacity must consist of elements whose average atomic weight is low. Compounds of hydrogen obviously will have the first place.

The most general way in which this fact of the high specific heat of water is important to life is the tendency of the sea, lakes, and rivers to prevent any considerable change of temperature. It also enables vast quantities of heat to be transported from the hotter to the colder parts of the earth by means of ocean currents. Naturally, other properties of water, such as latent heat of evaporation, etc., play a large part in maintaining a constant temperature.

The high specific heat of water is directly favourable to the living organism, composed as it is, in its active parts, of some 80 per cent. of water. The heat produced by muscular activity would otherwise cause a great rise in the temperature of the body before it could be eliminated from the surface by radiation and evaporation. The more highly organised a creature is, the more sensitive are the delicate adjustments of its chemical and physical processes to slight changes in temperature.

As L. J. Henderson points out (p. 91), the most striking change in modern laboratories

is the universal introduction of thermostats for carrying on investigations at a constant temperature. In fact, looking round my own laboratory recently, I noticed that there were five of these adjusted to various constant temperatures.

Finally, we note that the only other liquid exceeding water in specific heat is liquefied ammonia.

LATENT HEAT

Latent heat is the quantity of heat required to change the state of a solid to a liquid, or that of a liquid to a gas, at the same temperature; or that given out when the reverse change takes place.

In the case of water, 80 calories are necessary to convert 1 g. of ice at 0° into 1 g. of liquid water at the same temperature. This means that as much heat is required for this purpose as to raise the temperature of the resulting 1 g. of liquid from 0° to 80°.

To convert 1 g. of water at 100° to 1 g. of vapour at the same temperature, even more is wanted, viz., 536 calories; so that to vaporise 1 g. requires as much heat as to raise 536 g. by 1°.

A diphasic system of ice and water is therefore an extremely delicate thermostat. As heat is added or removed, no change of temperature takes place, merely ice is melted or water frozen. In this way, the temperature of large bodies of water never falls below their freezing points, and cannot do so, until the whole mass is frozen through.

The freezing point of water is not by any means a low one, compared with that of other liquids, and most chemical reactions can take place at this temperature. The latent heat of melting of ice, moreover, is greater than that of any other liquid except ammonia.

The latent heat of evaporation is more important still in the regulation of temperature. Unlike freezing, evaporation takes place at all temperatures, even below 0°. It is naturally greater at higher temperatures, and this fact, in itself, conduces to moderate a rise of temperature when it is already high, while having less effect when the temperature is low.

After what has already been said, it will not surprise the reader to find that the latent heat of evaporation of water is absolutely the greatest of all substances known, not even excepting ammonia.

It is to be noted that the large amount of solar heat absorbed in the vaporisation of water from the ocean is recovered again when condensation takes place as rain, and serves not only to warm the cooler places where condensation occurs, but as the source of all the water power of the earth. No other liquid could do this with the same economy of material.

The importance of evaporation in getting rid of the excess of heat produced in animal metabolism has been referred to above. If the surrounding temperature is the same as that of the organism, no loss can take place by radiation or conduction, so that evaporation is the only means available, but, at the same time, it is the most effective one.

CONDUCTION OF HEAT

Here again, water, although a poor conductor compared with metals, takes the highest place among other liquids and even non-metallic solids. The relative values in the following list will illustrate this point:—

Silver	1·00	Glass	0·0016
Lead	0·08	Glycerol	0·00066
Water	0·0125	Alcohol	0·00046

Thus there is more difference between silver and lead than between lead and water.

This fact has its importance in respect of the transference of heat between cells or parts of the same cell where structure prevents convection currents.

EXPANSION BY HEAT

The fact that water has its maximum density at a temperature of 4° above its freezing point is familiar to all. Unlike most common substances, when cooled from 4° to 0°, instead of contracting, it expands. At the moment of solidification, there is a further expansion, but this is not uncommon. The two phenomena together account for the fact that large bodies of fresh water, when cooled, freeze only on the surface. Since water at 4° is denser than at a lower temperature, it will sink and no ice will be formed in the depths until it has reached them by growth from the top. In salt water, of course, the ice that separates is free from salts and is therefore still lighter than the sea water.

If ice were formed in the winter at the bottom of lakes and streams, it would never get melted in summer, since the process of diffusion of the warmer and lighter water from the surface is so slow. An old experiment of Rumford's shows that a test-tube of water frozen at the bottom can be boiled at the top without melting the ice. In the lakes, the ice would become thicker every year, until ultimately the whole, or nearly the whole, of the water would be turned to ice.

So far for the thermal properties of water. The only other liquid which approximates to it in the merely thermal properties, necessary for life as we know it, is ammonia, and even this lacks the anomalous expansion before freezing.

L. J. Henderson (1913) makes use of these characteristics of water, and there are other exceptional ones, as we shall see, in order to illustrate his point of view that we must consider, not only the adaptation of the organism to the environment, but also the fitness of the environment to the organism. Of course, in one sense, the adaptation of the organism to a particular condition implies also that this condition is fitted for the organism, but there is an obvious distinction to be made, since the organism is capable of change in response to changes in the environment, while the converse does not occur. None the less, it is a remarkable fact that the properties of the substances everywhere present, such as water and carbon dioxide as also those of carbon itself, are just such as to allow the most varied and complex chemical and physical systems with which we are acquainted, and call by the name "vital," to be evolved. No doubt, the *crux* of the question lies in the words "call by the name vital." In a world in which liquid ammonia took the place of water, another kind of complex organisation might have been developed; although, it must be admitted, it seems impossible that the complexities and endowments of the "organisms" formed could ever reach the perfection of those which we know under the present conditions (see also the remarks on adaptation on page 201 above).

SURFACE TENSION

We pass on to consider some other of the physical properties of water. As we have seen, its surface tension, 75 dynes, is higher than that of any other liquid except mercury, although glycerol, 65 dynes, is not far below it.

We have also seen, in Chapter III., the importance of this in relation to the phenomena of adsorption, which play so large a part in physiological processes, owing to the heterogeneous nature of the systems concerned.

The supply of water from the soil to plants is greatly influenced by the large surface tension of water, since it is thus enabled to reach the roots from a considerable distance. It is said that, under ordinary circumstances, water may rise in the soil as much as 4 or 5 feet. See the monograph by Russell (1912, pp. 102-105).

TRANSPARENCY TO RADIATION

Water in the liquid state is practically transparent to all the rays of the visible spectrum. In very deep layers it appears blue, which means that it absorbs more of the rays of longer wave length than of the shorter. The rays of still longer wave length, heat rays, are comparatively more absorbed, so that a vessel of water is a fairly efficient method of absorbing the heat from an arc lamp, used for purposes of microscopic observation or photography. Ultra-violet rays are absorbed to a very small extent.

This relatively small absorption of the energy of radiation is probably of some importance in allowing the access of this form of energy to substances in solution in water. Especially in the case of the green leaf, the light energy must not be degraded to heat before reaching the photo-chemical system of the chloroplast.

AS A SOLVENT

When it is said that chemistry has been built up almost entirely on aqueous solutions, it is not to be understood that water has been used as a solvent merely because of its cheapness and accessibility, but that it has unique properties in this respect. In fact, there is no other liquid capable of dissolving so great a variety of substances. As regards inorganic salts, very few are soluble in any other liquid. Of organic substances, more are to be found which require alcohol, ether, and so on for solution, but, even here, the majority can be dissolved in water.

Geological facts are, perhaps, the most striking evidence of the efficiency of water as a solvent, but details are out of place here. It is sufficient to recall the fact (L. J. Henderson, 1913, p. 113) that the total amount of dissolved matter carried by the rivers of the world to the sea amounts to five thousand million tons per annum.

Turning to the living organism itself, a list of the substances found in urine, which were practically all previously in solution in the blood, illustrates the variety of chemical compounds soluble in water. These are:—urea, carbamic acid, creatinine, creatine, uric acid, xanthine, guanine, hypoxanthine, adenine, oxalic acid, allantoin, hippuric acid, phenaceturic acid, benzoic acid, phenolsulphuric acid, indoxylsulphuric acid, paraoxyphenylacetic acid, urobilin, urochrome, uroerythrin, hæmatoporphyrin, glucose, lactose (when the mammary glands are active), glycuronic acid, glycine, alanine, leucine, tyrosine, various enzymes, putrescine, cadaverine, chlorides, bromides, iodides, phosphates, sulphates, salts of potassium, sodium, ammonia, calcium, magnesium, iron, carbonic acid, nitrogen, argon and other substances. In pathological conditions: proteins, oxybutyric and acetoacetic acids, acetone and, in some abnormalities of metabolism, cystine and homogentisic acid. Only a few of these are soluble in other liquids to any extent, even in alcohol.

Chemical Stability.—With the exception of hydrolytic and electrolytic dissociation, the action of water upon solutes is practically nil. This depends upon its chemical inertness and stability. Substances can therefore be recovered, by evaporation of the solvent, in their original state. This applies also to substances which undergo electrolytic dissociation, since the ions reunite on concentration; and even to some extent to hydrolytically dissociated solutes, when the products are non-volatile.

Solubility.—As to what happens in the actual process of solution, we are, as yet, very much in the dark. Why, for example, sodium salts are nearly all soluble in water, whereas certain corresponding potassium salts are insoluble, and why the nitrates of practically all metals are freely soluble, but only the chlorides of some of them, is not explained. The fact itself is of great importance in the production of osmotic pressure. When dissolved, the molecules of a substance are free to manifest the effects of the energy due to their movement. The process is, in fact, a "dispersion" of the same kind as that more or less visible and obvious in the case of colloidal solutions, differing only in the degree of subdivision.

The history of the various theories proposed is of much interest and may be read in the account given by Walden (1910). We note that there has been much argument between the adherents of physical and of chemical theories. The question has, from the first, been closely connected with that of the nature of chemical affinity, so that as molecular physics made further and further strides, attempts were made repeatedly at physical explanations of chemical affinity. In the case of solution, as we shall see presently, there is undoubted evidence of combination of some kind between solvent and solute, "hydration" or "solvation"; but, since the chemical properties of a substance suffer little or no change in the process, it seems rather a matter of words whether we choose to consider the process as one of satisfaction of "residual affinities" or prefer to speak of attractive forces between molecules; neither, in fact, goes far towards an explanation and the different modes of expression serve equally well at present and will probably appeal differently to investigators according to whether they are chiefly occupied with the physical or chemical aspects of the phenomena. In the distant future they will, no doubt, be reconciled; although, presumably, it must be admitted that the explanation will most likely be in a better knowledge of the physics of the atom.

As dilute solutions are of frequent use in physiological work, and changes in their concentration require to be known, it may be useful to refer to the delicate

method of measuring these changes by the principle of *interference* of light waves. A method has long been in use for many purposes, in which the refractive index of a liquid is determined directly, by the amount of deviation through a prism; but the method by which changes in the refractive index are caused to produce interference bands is far more delicate. Suppose that we have a train of light waves, of a particular wave length, and that part of this passes through a column of water, on the one hand, and another part through a solution of a substance which slows the rate of transmission of light through it. The wave length will not be the same in the two beams, so that, if they are combined together, the direction of vibration, if coincident at one point, will be opposite at a certain number of waves distant, where there is half a wave length difference between them. When there is again a whole wave length difference, the directions are again coincident. The result is a series of alternate dark and light bands. This brief description is only intended to illustrate the principle on which the method is based. Details of the construction of the instrument will be found in Löwe's papers (1910 and 1912). It will be clear that the changes in concentration to be measured must affect one constituent of the solution only, unless those of other constituents are related to this in a known way. The method can also be used, as originally by Rayleigh, for the analysis of mixtures of gases, if the tension of one only varies independently. The instrument, as made by Zeiss, determines the concentration of solutions up to 8 per cent. sodium chloride with an error of 0·003 per cent. of the solute, or, with a longer chamber, solutions between 0 and 1 per cent. with an error of 0·0004 per cent. in the salt. The interferometer is also made by Messrs Hilger.

Hydration of Solute.—As just mentioned, there is, at all events in a large number of cases, combination of some kind or association between the molecules of the solvent and those of the solute. Leaving out for the present the hydration of ions, it must be admitted that the evidence for such hydration is mainly indirect, and, in fact, Nernst (1911, pp. 271 and 537) appears to regard the hypothesis as by no means proven.

The meaning of the name "hydration" must be distinguished from that of hydrolytic dissociation. The former refers to the combination of the molecules or ions of the solute with the molecules of water as such. The latter, as already explained, is a decomposition of a salt into free acid and base by interaction with the hydrogen and hydroxyl ions of electrolytically dissociated water.

The solubility of gases in water is diminished, not only by electrolytes, but also by some non-electrolytes, and the most satisfactory way of accounting for the fact is that the solute has in some way taken up a number of the molecules of the water, leaving fewer to dissolve the gas. One molecule of saccharose, for example, takes up six molecules of water (Philip, 1907). Carl Müller (1912, p. 502) finds that the diminution of solubility of a gas by a given solute is independent of the chemical nature of the gas. This can only be explained by an influence of the solute on the solvent, and most readily by the formation of "hydrates." This phenomenon of hydration may possibly play a part in the effect of neutral salts on the activity of an acid in the inversion of cane-sugar. It is clear that, if the neutral salt takes up a number of the molecules of the disposable water, the acid present will be in higher concentration in the remainder. It seems, however, doubtful whether this effect is capable of accounting for the whole of the apparent increase in the concentration of $H^{\cdot}$ ions (see also page 195 above).

Considerable evidence has been brought by Jones (1907) and by Jones and Anderson (1909) in favour of the hydration of salts in solution. If this takes place, it is generally supposed to be an equilibrium of such a kind that the more water present, relatively to the solute, the more molecules of it are associated with each molecule of the latter. Now, the absorption of light by solutions of substances is, by Beer's law (see Chapter XIX.), proportional to the number of molecules through which the rays pass. Further, if water molecules are taken up, it is to be expected that the vibration period and other properties of the molecules of the solute will be found to be different according to the dilution. Fig. 67 shows four series of photographs of absorption spectra of solutions of

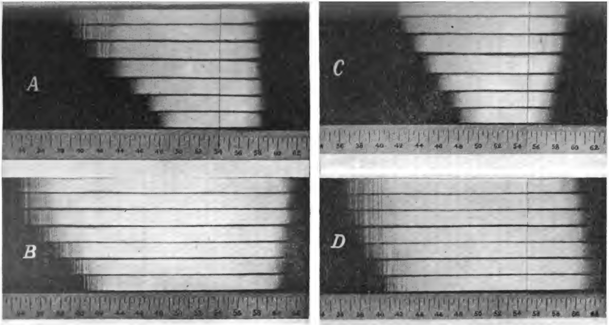


FIG. 67. ABSORPTION SPECTRA TO ILLUSTRATE THE HYDRATION OF SOLUTES.—Regions of absorption (black) in the blue and in the red, as indicated by the wave length scale, of solutions of copper chloride in water. The solution next to the scale is the most concentrated. The absorption by the same quantity of solute is, as shown, greater in the more concentrated solutions than in the dilute ones.

A, Concentrations from 4·5 to 0·562 molar.

B, Similar series with concentrations from 1·5 to 0·19 molar, in the same range of thickness of stratum as in *A*.

C and *D*, In these two sets of spectra the same depths of cell were used as in *A* and *B*, but the concentrations of the solutions were taken so that the number of *undissociated molecules* in the path of the light was constant. That is, the total concentration of the more dilute solutions was higher than that of the more concentrated, so as to compensate exactly for the increased electrolytic dissociation.

(Jones and Anderson, 1909. Photographs 30 and 31, Carnegie Institution of Washington.)

copper chloride in water. In A, the concentration increases from 0·562 molar to 4·5 molar, from above downwards, and the depth of the solution is varied inversely with the concentration, so that the same total amount of solute lies in the path of the light. It is seen that the more dilute the solution, the less ultra-violet is absorbed. In B, we have a similar series with more dilute solutions. In C and D, the concentrations are chosen so as to compensate for increased dissociation on dilution, so that the number of undissociated molecules in the path of the light should be constant. A similar effect is seen. There are two ways of accounting for the increase in absorption with concentration, when the number of molecules is kept constant. Aggregates may be formed and the absorbing power increased thereby; or solvates may be formed, in proportion to dilution, and the absorbing power decreased with increase in number of molecules of water taken up. To decide between the two views, we can test the effect of rise of temperature, which breaks up aggregates. The effect is the same as that of *increasing* concentration; hence it is to be concluded that the action of increased concentration on the absorption of light is not due to aggregation of solute, which would have the opposite effect. The concentration of water, in the experiments in question, was also varied by the addition of calcium chloride or alcohol, and the salts of several different metals were investigated, with results similar to those mentioned.

An important case for the physiologist is the state of amino-acids in water. Winkelblech (1901, p. 590) points out that taurine (amino-ethyl-sulphonic acid) forms no salt with hydrochloric acid, and that it is usually supposed to form a ring compound, internal anhydride, or internal salt, in water. Might it not also be that the sulphonic acid group makes it too strong an acid, even when partially counteracted by the NH_2? In the case of the ordinary carboxylic amino-acids, even supposing that such internal salts are formed by combination of the NH_2 and COOH groups with one another, as salts of very weak acids and bases, they will be greatly dissociated hydrolytically in water, according to the laws given on page 198 above. In fact, as Winkelblech shows (1901, p. 592), glycine, according to the equation of Arrhenius, must be hydrolytically dissociated to the extent of 99·967 per cent.; this proportion is present as hydrated glycine, the smaller remainder as internal salt together with a few ions. It is therefore present in solution practically entirely as—

$$\mathrm{CH_2}\begin{array}{l}\diagup\mathrm{NH_3\,OH}\\ \diagdown\mathrm{COOH}\end{array}\qquad \text{not as } \mathrm{CH_2}\begin{array}{l}\diagup\mathrm{NH_3}\\ \diagdown\mathrm{COO}\end{array}$$

It is difficult to see, however, why the first compound is not dissociated more completely than it is found to be. Possibly the explanation lies in the production of an internal ammonium salt (see page 202 above).

The fact that taurine and the corresponding carboxylic acid, alanine, have the same very small electrical conductivity shows that electrolytic dissociation is extremely low; one would expect that the presence of the strongly acid sulphonic acid group would give rise to considerably more $H^{\cdot}$ ions than the carboxyl group. This is one of the numerous cases that show that the chemical properties of a particular group are not fixed, but depend on other constituents of the molecule.

The relation of *lyophile colloids* to the solvent has been treated of above (page 97).

DIELECTRIC CONSTANT AND ELECTROLYTIC DISSOCIATION

In the previous chapter the relation of the dielectric constant to electrolytic dissociation has been discussed and the fact pointed out that water has a higher dielectric constant than any other solvent, with the exception of prussic acid and hydrogen peroxide. Even where electrolytic dissociation is produced by other solvents, the process appears to be a very complex one compared to the simple splitting of the majority of salts in water. Association of solvent and solute seems to occur to a large extent, as well as between the molecules of the solute itself. Electrolytically dissociated colloids in water (page 160) are similar cases.

Although water does not chemically decompose salts dissolved in it, yet by causing their dissociation into ions, it enables all kinds of reactions to take place which do not occur between the solutes in their molecular state. It was shown by Veley (1910, p. 49) that pure nitric acid does not react with calcium carbonate. The importance of ions in physiological processes has been abundantly illustrated in the previous chapter and need not be further insisted on here.

If now we look at a series of substances arranged in order of dielectric constants, heats of vaporisation and conductivity for heat, we notice that there is an unmistakable connection between these properties. It will also be found that these properties are related to the critical pressures and to both the constants of van der Waals. So that, after all, some of the wonderful properties of water are mutually dependent.

THE CONSTITUTION OF WATER

The actual percentage composition of water, as formed by two volumes of hydrogen to one of oxygen, was proved by Cavendish (1781), although the true explanation of the results obtained was not known until the experiments of Lavoisier in 1783, as Cavendish held to the doctrine of phlogiston.

It is only of recent years, however, and owing greatly to the influence of Armstrong, that it has been realised that water cannot be correctly represented by the symbol H_2O, with the molecular weight of only 18, or rather it is only under limited conditions that this can be done.

In the first place, the freezing and boiling points are not at all where they would be expected to be in a simple compound containing three molecules only of gases with extremely low freezing and boiling points. In fact, comparing it with similar compounds, as Jacques Duclaux points out (1912), the freezing point should be about $-150°$ and the boiling point $-100°$. It appears then that the molecular weight of water must be greater than 18; in other words, it must be a polymerised or associated liquid, in which a number of molecules are united together. Comparing formaldehyde, which is liquid at $-20°$, with its polymer trioxymethylene, composed of three molecules of formaldehyde, we notice that the latter is solid even at 150°; so that considerable changes of properties occur even when only three molecules are combined together, and although H_2O ought to boil at $-100°$, H_6O_3 might well boil at $+100°$.

We must suppose that chemical combination takes place between the simple molecules when polymerisation takes place. Thus, although formaldehyde and glucose have the same percentage composition, no one would regard them as the same chemical substances. Also, at any given temperature, there is an equilibrium between the polymers of water, which are mutually convertible, so that the different chemical individuals are easily changed into one another, and the chemical change is by no means so marked as in the example given above.

We may now at once proceed to make use of the names proposed by Sutherland (1900). The substance composed of single molecules, which does not appear to exist as a liquid, is *hydrol*, that of two molecules is *dihydrol*, that of three molecules is *trihydrol*, and so on.

So far the theory is simple, but already several of the peculiar properties of water can be explained by it. The degree of polymerisation, as a general rule, increases as the temperature falls, so that cold water is not the same liquid chemically as warm water and is less volatile; hence its vapour pressure falls more rapidly than that of a simple liquid would. This is a favourable circumstance in regard to the properties of water as a regulator of animal temperature, since the cooling produced by its evaporation is greater the higher the temperature is.

We saw that the specific heat of water is unusually high. Now when heat is applied to water, it has to do three things: a part serves to heat the complex molecules, another part to heat the simple molecules, and a third part to decompose a certain number of complex molecules into simple ones. The specific heat of water, furthermore, presents a minimum at about 30°. The two first-mentioned fractions of the heat probably increase regularly with the temperature, as is usual, but the third rapidly decreases, being proportional to the concentration of complex

molecules present, which diminishes considerably between 0° and 100°; a minimum would therefore be expected.

There are, however, certain properties left unexplained by the hypothesis in this simple form. Röntgen (1892), considering what might be the nature of the polymer formed at low temperatures, was struck with the idea that it ought to show itself when the whole of the water was transformed into the polymer. But when water is cooled it turns into ice. How then do the properties of ice coincide with the requirements of the case? Take the density; ice is more bulky than water at 0°, so that if we assume that ice molecules exist in liquid water, we can explain the existence of a point of maximum density at 4°. Thus: the change of volume when water is warmed from 0° to 1° is the result of two opposite effects—dilatation of the simple molecules, according to rule, and contraction, due to change of ice into water. The latter process is preponderant at the lower temperatures, but nearly absent at the higher, and a point will exist where the difference between the two is the least. It will probably occur to the reader that water, according to this view, is a colloidal solution of ice. We shall see presently that a third component has to be added, namely steam.

Since the presence of the large molecules of ice increases *viscosity*, we see why this property of water increases unusually rapidly when the temperature falls.

The *compressibility* behaves similarly, on account of the effect of pressure in causing depolymerisation. This would of itself result in a diminution of volume and be added on to the compressibility of the pure hydrol.

There still remain some questions unanswered. Although the compressibility of water is greater than that of hydrol, it is unusually small. Again, we have not yet an explanation for the high dielectric constant, nor why ice is lighter than water.

There is an interesting fact in connection with water which throws some light on all of these problems. Water of all known liquids (except fused metals) contains the largest number of molecules per unit volume. Thus, in gram-molecules per cubic centimetre:—

Water	55	Hydrofluoric Acid	49
Bromine	20	Benzene	11·5
Sulphuric Acid	22	Heptane	7·1
Ammonia	37		

This means that there is less space between the molecules of water than of other liquids. The low compressibility is doubtless explained by this. The dielectric constant also increases rapidly as the molecular condensation of a substance increases. Finally, it is to be supposed that the molecular forces, which permit the molecules of hydrol to press unusually closely together, disappear when the new group constituting ice is formed, so that the latter occupies the greater volume corresponding to that which might be called the normal volume of water. It is to be admitted, nevertheless, that the reason why water is such a *closely packed liquid* has not been explained.

As to the actual number of molecules existing in the various polymers, opinion is still divided. The balance of evidence appears to be that ice is trihydrol, steam is monohydrol, liquid water is mostly dihydrol with varying amounts of the other two polymers according to the temperature. A curious fact is that, according to Nernst and Levy (1909), there are still some polymerised molecules in water vapour, so that, if these are identical with ice, it seems that we must admit the presence of ice in steam!

There is also difference of opinion as to the relative number of molecules of ice present in liquid water at various temperatures. As J. Duclaux (1912) points out, it might be possible to attack the problem by the determination of the absorption of light of different wave lengths by water and by ice. It appears that ice is much bluer in colour than water, which is stated to have, as dihydrol, a very pale green colour. The reader will probably have noticed that the ice of glaciers is of a deeper blue than that of the same depth of water.

It was incidentally mentioned above that it is necessary to introduce steam, as a *third component*, into the water system, so that water in its ordinary liquid state is a ternary mixture. This has been shown by Bousfield and Lowry (1910) by comparison of the properties of water with a series of aqueous solutions of which it may be regarded as the limit of dilution. The careful study of "solution volumes" of caustic soda at different concentrations and temperatures showed that, in addition to the abnormality of water near the freezing point, there is a second in the neighbourhood of 60° and that the factor responsible for this effect becomes more and more obvious as the boiling point is approached. The complete evidence

for the view that the phenomenon is due to the existence of a third compound, steam or monohydrol, is too long for the present work. One or two main facts should be given on account of their importance.

The "*Solution Volume*" of a given solute is the increase in volume of the solvent when 1 g. of the solute is dissolved in 100 c.c. of the liquid. Thus, when 1 g. of sodium chloride is dissolved in 100 c.c. of water, the volume of the solution is 100·2. c.c., so that 0·2 c.c. is the solution volume of 1 g. of sodium chloride. It might be supposed that this would be the volume of the salt in the liquid state, but this cannot be so, since the volume changes with concentration. Moreover, sodium hydroxide has a negative solution volume at certain temperatures and concentrations, so that 140 g. of the solid can be added to a litre of water at 0° without increasing the volume at all, keeping the temperature at 0°, of course. It is evident that changes take place in the solvent itself.

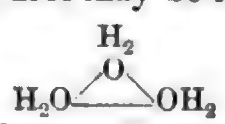
The contraction produced on dissolving is greatest in presence of large excess of the solvent, just as the number of molecules of water in the hydrated solute is greater the more dilute the solution. The most reasonable explanation of the contraction is, then, that the combined water has a greater density than normal water; a view indeed supported by other evidence.

Further, the degree of contraction with the same volume of solvent varies with the temperature, but in such a manner as to show a maximum at a particular temperature, which itself naturally varies with the degree of hydration of the solute used. In most cases investigated by Bousfield and Lowry, the temperature at which this maximum occurs is about 60°. On passing from solutes with a small affinity for water to those with a strong one, the maximum is reached at lower and lower temperatures; in the case of lithium chloride at 35°. The deviations thus have their origin at the higher temperatures and extend gradually downwards.

Now, in the case of the point of maximum density of water at 4°, we have seen that the most satisfactory explanation rests on the presence in liquid water of a polymer, identical with ice, which diminishes in concentration as the temperature rises. Similarly, to explain the changes in the volume of the water taken up in hydration of solutes, it is in accord with all facts to assume that, as the temperature rises, there is an increasing formation of a third component of low density, and a partial destruction of this when a hydrate-forming salt is added. It is natural to regard this third component as being identical with steam, that is, monohydrol, and, if this is so, the component intermediate between steam and ice must be dihydrol.

To sum up, we arrive at the conclusion that liquid water is a system of three components—ice, or trihydrol, which is present in greatest concentration at the freezing point; dihydrol, the main component at ordinary temperatures; and monohydrol, or steam, increasing as the temperature rises to the boiling point. It is to be remembered that, at any temperature, there will be a certain definite relative proportion of all three of these substances, although at the freezing point monohydrol is probably nearly absent, while trihydrol is nearly absent at the boiling point.

It is probable, as already remarked, that these three constituents must be looked upon as distinct chemical individuals, although easily converted into one another by small changes of conditions. Thus, regarding the quadrivalence of oxygen as an established fact, trihydrol may be represented :—

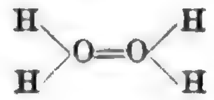


dihydrol : $H_2=O=O=H_2$

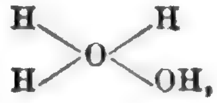
and in monohydrol, H_2O, two of the affinities of oxygen must mutually satisfy one another.

Armstrong (1908) prefers the name "hydrone" instead of "hydrol" to express the simple molecule and dihydrone, etc., for the polymers. The reason is that water belongs not to the class of alcohols, but rather to that of the ketones. Strictly speaking, this is no doubt correct, but, on the other hand, water may conveniently be regarded as the simplest of the alcohols, if we consider OH as the characteristic group of the class.

Armstrong assumes further that there is present in water an isomeric form of dihydrone, in which one of the molecules is resolved into H and OH, with increased chemical activity. Thus, dihydrone being



hydronol is :—



and, in this latter compound, we may consistently use the termination—ol. The activity of "hydronol" corresponds, on the "association" theory of chemical change, to the $H^{\cdot}$ and OH' ions of water on the electrolytic dissociation theory. In the former view, which cannot be discussed further in this place, the electrical conductivity of concentrated solutions, say of hydrochloric acid, is conditioned mainly by *hydrolysed* solute, $H_2O\langle{}^{H}_{Cl}$, and in dilute solution by *hydrolated* solute, $HCl\langle{}^{H}_{OH}$; so that, in strong solutions, it is chiefly the solute which is active, in weak solutions, the solvent. It follows further that "hydration" may be of two types, "hydrolation" and "hydronation." For more details the reader is referred to the paper quoted.

With regard to the actual existence of these two isomeric forms of the associated molecules of water, it is clear that they can be represented by structural formulæ; but, as previously remarked, this does not in itself prove their existence. I cannot pretend to be able to give an opinion on the evidence for this, about which there is much contention. I would merely point out that the phenomena whose explanation requires their assumption can, apparently, be explained as satisfactorily on the electrolytic dissociation theory.

In any case, the arguments of Bousfield and Lowry (1910, p. 18) are not affected, since, as they indicate, dihydrol, and perhaps trihydrol, would only have to be thought of as mixtures of hydrone and hydronol with a given average density, instead of simple substances.

It is important to note that Philippe A. Guye (1910), approaching the problem from the chemical point of view, also comes to the same conclusion as Bousfield and Lowry do, with regard to the ternary nature of water.

HYDRATION OF IONS

The behaviour of ions as regards combination with water is similar to that of solutes in general. The fact has been referred to in previous pages in various connections, so that it is unnecessary to discuss the question further, except to call attention to an interesting paper by Kohlrausch (1902). This investigator found that the rates of migration of different ions approached nearer to the same value as the temperature was raised. Above the normal boiling point of water the effect is still more obvious, as appears from the following table of Noyes and his co-workers (1907, p. 47):—

RATES OF MIGRATION OF IONS AT DIFFERENT TEMPERATURES.

	18°	100°	140°	156°	218°	281°	306°
KCl	130·1	414	565	625	825	1,005	1,120
NaCl	109·0	362	500	555	760	970	1,080
Ratio	1·194	1·144	1·130	1·126	1·086	1·036	1·037

If drawn in curves, these results show that the mobilities would be identical at 360° C., that is practically at the critical temperature of water. At low temperatures, therefore, the sodium ion is the more bulky and for that reason the slower in movement, on account of the fact that it has more water molecules associated with it than the potassium ion has. But at high temperatures, owing to the loss of water, the two approximate to equal size and mobility.

OSMOTIC PRESSURE, HYDRATION AND THE CONSTITUTION OF WATER

It might perhaps be supposed that the considerations of the previous pages would invalidate conclusions with regard to osmotic pressure, since the concentration of the solvent is diminished by the amount of it which is taken up by the solute, so that the effective concentration of the solute would be increased. It is pointed out by Nernst (1911, p. 271) that it is not found experimentally that any anomalies result from this cause.

The reason will be apparent from the following table given by Bousfield and Lowry (1910, p. 21):—

Molar Concentration of KCl.	Free Water in Grams per 1,000 Grams.	Water Combined with KCl in Grams per 1,000 Grams.
0·201	971	29
0·151	977	23
0·100	984	16
0·076	987·5	12·5

It will be noted that the proportion of water of hydration to total water diminishes rapidly with the concentration and that it is only in the high concentrations that it would be detectable, owing to the very small amount of water taken up at the lower concentrations. Even in 0·2 molar concentration 97 per cent. of the water is free.

Osmotic pressure, on the kinetic theory, being dependent on the energy of movement of the molecules of the *solute*, it is clear that a certain degree of polymerisation of the solvent, by which the total number of its molecules is decreased, will not have any obvious effect on the osmotic pressure of the solute.

ELECTROLYTIC DISSOCIATION OF WATER

The more carefully water is purified, the less is its power of conducting an electric current; so that the conclusion must be made that it is, at the most, only very slightly dissociated into ions. $H^{\cdot}$ and OH', therefore, can only exist beside one another in the merest traces. We have seen above the importance of this fact in the process of neutralising a base with an acid, and how, in consequence, the heat of neutralisation of a strong acid by a strong base is the same, whatever the acid or base used.

Now, since the conductivity of ordinary distilled water is readily shown to be due to impurities, it would seem that the view taken by Armstrong, that if water were sufficiently pure it would be a non-conductor, is a justifiable one. It is obvious that the argument cannot be disproved by direct measurements of the conductivity of purified water. At the same time, the results of Kohlrausch and Heydweiller (1894) distinctly point to a limit, beyond which further purification has no effect. These experiments give a concentration of $1·05 \times 10^{-7}$ gram-ions per litre at 25°, or $0·78 \times 10^{-7}$ at 18°. This gives a value for the product of ionic concentrations $(H^{\cdot})$ (OH') of $1·1 \times 10^{-14}$ at 25°.

The substantial correctness of the view, moreover, is shown by the fact that other independent methods give values almost identical with this.

1. The addition of large quantities of a strong alkali to water will render infinitesimal the concentration of any free hydrogen ions arising from the dissociation of an acid present as impurity; so that, if the presence of any such ions can be detected, they must arise from the water itself. This can be done by taking the electromotive force of a battery of acid and alkali by the method of Nernst described above (page 191). The value of the concentration in hydrogen ions found in this way was $0·8 \times 10^{-7}$ at 19°.

2. We have seen how satisfactorily the hydrolytic dissociation of certain salts in water is explained by the existence of $H^{\cdot}$ and OH' ions in water. This is, in itself, evidence for the truth of the hypothesis, but the numerical value of the dissociation of water can be calculated from the degree of hydrolysis of a solute and $0·68 \times 10^{-7}$ has been found in this way.

3. In the chapter on "Catalysis" we shall see how acids cause an increased rate of hydrolysis of esters in water and how alkalies cause an increased rate of saponification. So that, if the rates of these reactions in pure water be determined, we have another means of arriving at the concentration of hydrogen or hydroxyl ions in water. Taking methyl acetate, Wijs (1893) found a value of $1·2 \times 10^{-7}$ at 25°.

Putting the four values together and converting them to the same temperature (25°), we find :—

By acid-alkali battery - - - -	$1{\cdot}19 \times 10^{-7}$.
By hydrolytic dissociation of solute - -	$1{\cdot}10 \times 10^{-7}$.
By saponification of esters - - -	$1{\cdot}20 \times 10^{-7}$.
By electrical conductivity - - - -	$1{\cdot}05 \times 10^{-7}$.

It is impossible to believe that values so near together could depend on accidental impurities.

It should be remembered also that Arrhenius (1889, p. 103), on this hypothesis, was enabled to predict the high temperature coefficient of its conductivity.

On the other hand, Walden (1910) finds that water has no higher conductivity when dissolved in prussic acid, contrary to binary electrolytes of the ordinary kind. It seems, however, that there are anomalous conditions present, owing to chemical combination with the solvent.

Water, as Nernst points out, is capable of a second electrolytic dissociation, since

$$OH' \rightleftarrows O'' + H^{\cdot}$$

But the separation of the second hydrogen ion from such a dibasic acid always takes place with great difficulty, so that the concentration of oxygen ions would probably be so small as to escape detection.

HYDROLYTIC DISSOCIATION OF SOLUTES

There are a few more facts in connection with this question which require mention.

Denham (1908) has shown that the hydrogen electrode can be used with good results in determining the *degree of hydrolysis*. The most interesting facts, for our purposes, obtained in this way are that ammonium chloride is only hydrolytically dissociated in water to a minute extent, namely, 0·018 per cent. for a 0·01 molar solution at 25°, while aniline hydrochloride 0·031 molar is 2·6 per cent. dissociated, whence ammonium is about seventy thousand times as strong a base as aniline.

The *hydrolytic dissociation of Indicators* is of importance as showing that their strength as acids or bases must not be too small; otherwise the end point is inaccurate. The rule is that weak bases and weak acids are not to be used together; that is, weak acid indicators are not to be used for titrating weak bases, nor weak bases for titrating weak acids. For more details see Nernst's book (1911, p. 535).

The fact that hydrolysis can be reduced by the addition of excess of acid or base, respectively, enables precipitations to be avoided where the product of hydrolysis is insoluble. Thus acetic acid is added to mercuric acetate. Conversely, by reducing the $H^{\cdot}$ ion concentration in ferric chloride solutions by the addition of sodium acetate, ferric hydroxide is precipitated. Or silicic acid may be precipitated from sodium silicate by addition of ammonium chloride. This kind of action is obviously of much importance in the reactions of analytical chemistry (Nernst, 1911, p. 548).

Finally, the circumstance that, when the weak base or acid of a hydrolytically dissociated salt has a very small conductivity, it is found that addition of excess of this component beyond a certain degree causes no further change in the molar conductivity of the solute, as shown by Bredig (1894, 1, p. 214), enables the degree of hydrolysis to be determined by an independent method. Such a case is that of aniline salts.

WATER AS CATALYST

The phenomenon known as "catalysis" will come up for discussion in a later chapter. It will suffice here to state that there are substances which produce a great increase in the rate of reactions, although they themselves are not constituents of the final system in equilibrium and, as a rule, reappear finally in the same state as they were to begin with.

Hydrogen ions constitute one of the most powerful of these catalysts and,

although they exist only in very small amount in water, their action must not be neglected.

Hydroxyl ions are not catalysts, at all events in the saponification of esters, since they are used up in the reaction, thus:—

$$CH_3CO_2CH_3 + OH' = CH_3COO' + CH_3OH.$$

The result of this reaction is to increase the hydrogen ions and to diminish the hydroxyl ions. There is, then, a double process, the details of which may be found in Nernst's book (1911, p. 567).

Most oxidation processes were supposed, up to recent times, to be simply explained by the direct union of oxygen with the substance to be oxidised; but it has been shown conclusively, chiefly by the work of H. B. Dixon and H. B. Baker, that the presence of water is necessary. This fact will require further discussion in our chapter on oxidation, so that attention is directed to it here as another case in which water acts as a catalyst.

It should be mentioned that Armstrong does not admit that it is water itself which acts in these cases, but the impurities contained in it, acting as conducting systems to bring the other components into reaction. A striking case, which seems to support this view, is that described by Brereton Baker (1902). It had been already shown by Dixon that water vapour is necessary for the explosion of a mixture of oxygen and hydrogen gases. Baker showed that if the gases are almost completely dried, a slow combination occurs on heating; but although more than sufficient water is formed to bring about an explosion, none happens. The explanation, according to Armstrong, is that the water formed is too pure to allow the necessary conducting system between the reacting gases to be produced.

WATER IN REVERSIBLE REACTIONS

A large number of the reactions occurring in living organisms are those in which water is removed or added. The addition of water, hydrolysis, results in the splitting up of a complex molecule into smaller ones, and plays a large part in the phenomena of digestion, where certain agents, enzymes, are present whose function it is to hasten the process catalytically. As a simple instance, we might take glycyl-glycine:—

$$COOH—CH_2—NH—CO—CH_2—NH_2.$$
$$\uparrow$$

By the entrance of a molecule of water at the arrow, the compound is split into two molecules of glycine:—

$$COOH—CH_2—NH_2 \qquad HOOC—CH_2—NH_2.$$

If two molecules of glycine be taken and a molecule of water removed, that is, H from the one, and OH from the other, synthesis of glycyl-glycine occurs.

Consider, further, the equilibrium in a mixture of methyl acetate and water. Here, when water is added to methyl acetate in the proportion of one molecule to each molecule of the ester, part of the water hydrolyses part of the ester similarly to the previous case. But, when a certain fraction of the ester is hydrolysed, the process comes to an end, owing to the increase of the opposite synthetic reaction by mass action of the products of hydrolysis. Expressed in the usual way, we have in equilibrium:—

$$K . C_{ester} . C_{H_2O} = C_{alcohol} . C_{acid}, \text{ or } K = \frac{C_{ester} \times C_{H_2O}}{C_{alcohol} \times C_{acid}}$$

Suppose that we now increase the concentration of the water. It is plain that the only way K can remain constant is by diminution of C_{ester}, which involves, at the same time, increase of the components of the denominator. Similarly, decrease of water means increase of ester, or synthesis. It is clear that, in this way, by alteration of the actual or effective concentration of water, the living cell has the possibility of changing the position of equilibrium in such reversible reactions, and thus causing the preponderance of hydrolysis or synthesis. It seems most probable that mechanisms of such a kind are active in the protoplasmic system, and that the taking up or giving off of water by colloidal substances is the chief one. In any case, we see the importance of the presence of water, not merely as a solvent to allow the reagents to come together, but also as an actual component of the chemical reactions themselves.

In pure water, the process of attainment of equilibrium is extraordinarily slow,

so that it must be hastened by a catalyst. Hence the universal presence of enzymes in the organism.

Although water, as such, is chemically so inert a substance, certain chemical individuals, such as sodium, enter into violent reaction with it. Here again, however, we are met with the possibility that the reaction is accelerated, or even rendered possible, only by the presence of some other substance, which acts as a catalyst. H. Brereton Baker (1910) and Baker and Parker (1913) have shown how greatly the rate of reaction between sodium amalgam and water is retarded by purification of the water.

DRYING AND STERILISATION

The necessity of the presence of water for the manifestation of vital properties is sufficiently obvious from the former part of this chapter. An interesting question arises as to how far protoplasm can be deprived of water, while remaining capable of recovery to life, when again supplied with moisture.

That drying in ordinary air is not necessarily fatal is shown by every-day experience with seeds, which can be kept a large number of years without losing their power of germination.

Shattock and Dudgeon (1912) have shown, moreover, that certain bacteria, even when they do not produce spores, can be exposed to a vacuum, produced by charcoal surrounded by liquid air, for a space of one hundred and sixteen days. One would suppose that all water would be removed from the organisms in this way. Mr Shattock informs me, that he has found, since the paper referred to was published, that after two years in the vacuum, *Bacillus pyocyaneus* was still capable of vigorous growth.

Apparently, under such conditions, all chemical processes cease, so that we must assume that the protoplasm remains in the state in which it was at the moment of desiccation and prepared to resume activity on the arrival of water. It is interesting to note that the bacillus in question lives longer in the dry vacuum than when merely air dried; in this latter state it never survived longer than nine days, no doubt owing to chemical changes still continuing. Some kind of change can be brought about, even in the perfectly dry condition, since, if exposed to sunlight or ultra-violet radiation, it was found by Shattock and Dudgeon that bacteria were killed rapidly, even in the absolutely dry vacuum.

Naturally, the much more complex and sensitive organisation of the higher animals cannot be dried in this way. It is well known, however, that creatures as highly developed as Rotifers survive drying in air; but this appears to be due to the production of a capsule which prevents complete loss of water. Davis (1873) saw a drop of fluid exude when he punctured the cyst of Philodina.

It seems possible that desiccation at the eutectic temperature by Altmann's method, described in the first chapter of this book (page 17), might allow of recovery of the cells of higher organisms. If so, a valuable means of investigation would be available; tissues, dehydrated in this way, can be cut into thin sections and the cells observed under the microscope. The difficulty, as previously mentioned, comes in when it is required to add water again.

An important practical application of the facts described above, as to the necessity of the presence of water for protoplasmic activity, lies in the greater resistance of organisms to the action of heat the drier they are. This is, however, not invariably the case—*Bacillus pyocyaneus* is killed by exposure to 65° for an hour, wet or dry. The resistance is particularly noticeable in the case of spores of bacteria and other fungi; as is well known, a higher temperature of sterilisation is required to kill them. This behaviour is also shown by enzymes, which resist a considerably higher temperature in the dry state than when in solution.

A fact worth recording here is that, as shown by Dreyer and Ainley Walker (1912), spores of bacteria suspended in glycerol or oil are not killed by exposure to a temperature of 119° C. for over half an hour. This fact is obviously of much practical importance, since sterilisation in non-watery liquids is frequently made use of.

That organisms are under more or less risk of injury from drying is shown by the precaution taken by many of them to avoid the risk by surrounding them-

selves with a layer of substance comparatively impermeable to water, forming what are known as "cysts," as mentioned above in reference to Rotifers.

It will also readily be understood that, in the dry state, protoplasm can withstand freezing temperatures better than in the normal active moist state. Seeds, although they are not absolutely devoid of water, can be exposed to the temperature of liquid air without injury.

HYDROTROPISM

The need of water causes certain organisms to turn towards the place where it is to be found. This fact is very marked in the case of roots, leading to the phenomenon known by the above name. The side of roots turned away from the water grows more rapidly than that turned towards it, so that curvature results. The opposite behaviour is shown by the sporangia of Mucor, leading to bending away from the moist surface.

THE VISCOSITY OF LIQUIDS

The subject of viscosity is, strictly speaking, not quite in place here, since it concerns other liquids in addition to water. But since, in physiological work, the liquids with which we have to deal are, almost entirely, solutions or suspensions in water, we may be allowed to take the subject at this stage, as a convenient one.

As was pointed out by Newton, the particles of liquids are not free to move about without resistance due to their "adherence" to one another. This gives rise to friction, so that the viscosity, or internal friction, of a liquid is proportional to the velocity with which these particles are moving past one another and also to the extent of the rubbing surfaces.

The *methods* used for its determination consist either in measuring the resistance offered to the movement of a surface passing through the liquid, or in that of the resistance offered to the passage of the liquid through a narrow tube; the latter method is a simple one and requires merely the determination of the time taken by a given amount of the liquid, under a given pressure, to run through the tube.

The flow through tubes is not only the most important aspect of this property of liquids met with in ordinary life, but also in physiology, where the internal friction of the blood gives rise to what is often called the "peripheral resistance" of the vascular system. This it is, that, with a given rate and strength of heart beat, determines the arterial pressure.

The first point to be noted is, that when a liquid is being caused to flow through a tube by the pressure applied at the inlet end of the tube being greater than that at the outlet, the layer in immediate contact with the wall of the tube is at rest, while that in the middle has the greatest velocity; each layer experiences friction at its contact with the neighbouring layer, so losing in velocity progressively until the outermost layer is reached, where the velocity disappears entirely. We see, then, that the friction is between the parts of the liquid itself and not between the liquid and the wall of the tube.

Suppose, next, that the tube is a wide one and that the internal friction of the liquid is not great; the thickness of the layer at the periphery in which the velocity is increasing from zero to its maximum rate will only be a narrow one The remainder of the column moves in all its parts with the same velocity, so that, in this part of the stream, there is no friction. Such tubes are the large arteries and veins. In a narrow tube, such as an arteriole, the layer whose constituent elements are in motion relatively to one another will reach to the axis of the tube, so that the whole of the liquid column is exposed to internal friction. We see, then, how, even supposing that the number of arterioles into which a large artery divides is sufficiently great to give a total cross-sectional area equal to that of the large artery, so that the rate of flow is no greater, the total mass of blood is causing frictional resistance, whereas in the large

artery merely a small fraction of it was doing so. The sectional area of the arterioles taken together may clearly be even greater than that of the artery, without affecting the nature of the result, although the effect will be less, on account of the less rate of flow.

It is important to bear in mind that the peripheral resistance of the arterial system, resulting from the division into small arterioles, is due entirely to the internal friction of the blood, not to friction against the walls of the vessels; except indirectly, in so far as it is this latter friction which determines the stationary condition of the blood film in contact with them.

Frictional resistance in fluids being proportional to the rate at which the rubbing surfaces glide over one another, we see why there is comparatively little resistance in the capillaries. Owing to the enormous increase of total sectional area, the rate of flow is far less than in the arterioles.

Now, the total amount of the friction experienced by the blood obviously depends on that property of liquids known as internal friction, which differs greatly in different cases; compare water with treacle, for example. It is, therefore, of some importance to find out what are the various conditions on which this property depends.

We have to consider homogeneous liquids, such as pure liquids and true solutions, colloidal solutions and suspensions, such as that of blood corpuscles in plasma.

The coefficient of viscosity is defined as the force per unit area required to produce a difference of unit velocity of streaming in two parallel layers one centimetre apart. It is $6{\cdot}6 \times 10^{-3}$ dynes in the case of water, about 10×10^{-3} for serum, and 25×10^{-3} for blood.

Chemical Composition.—As a rule, the internal friction increases with the molecular weight and, in homologous series, in proportion thereto. The increase of the viscosity of water, due to the formation of polymers of a higher molecular weight, has been discussed above.

Temperature.—Rise of temperature causes considerable decrease of viscosity, as is known to every one in the case of such liquids as castor oil, glycerol, etc. Hence also the necessity of using a thick lubricating oil for the cylinder of an air-cooled petrol motor; the high temperature would make another one too thin to serve its purpose. The viscosity of blood diminishes to a large extent as the temperature is raised, so that less work is demanded of the heart in order to drive a given amount of blood through the arterioles; or the same work will drive the blood at a greater rate. This is an incidental advantage possessed by warm-blooded animals.

Blood.—Changes in the viscosity of blood, other than those produced by differences of temperature, are also of importance. The presence of corpuscles increases the viscosity, which is therefore lower in "laked" blood than in normal blood. Dilution has also the effect of diminishing viscosity, so that a dilute blood passes more rapidly through the renal vessels and the excretion of urine is favoured. See the papers by Denning and Watson (1906) and Burton-Opitz (1911).

Blood is a suspension and should obey the logarithmic law proposed by Arrhenius (1917). If x is the viscosity, relative to that of the liquid phase, of a suspension in which c is the percentage of volume occupied by the suspended particles, and θ a constant,

$$\log x = \theta c.$$

Bazett (1919) finds that if θ be given the value $0{\cdot}00773$, obtained from a particular case, the formula gives values for dilutions of blood with plasma which correspond to the experimental data. Multiplying these figures by the viscosity of the liquid phase (plasma), we have the viscosity of the suspension.

Viscosity of Colloidal Solutions.—The internal friction of the blood-plasma, as a colloidal solution, is affected by the same factors as those which act on that of colloidal solutions in general. A brief account only can be given here; the reader will find more details in the report of the discussion at the Faraday Society on 13th March 1913. As regards suspensoids, the degree of dispersity is the main factor, and it appears that the maximum of viscosity is at medium values of dispersion, being less with very small as well as with

very large particles. It is uncertain whether this is connected with the variable amount of the dispersion medium associated with the particles. Emulsoids show great variety of changes in viscosity, so that the determination of this property is a valuable one in the investigation of such systems (see the paper by Wo. Ostwald, 1913, from which the following statements are chiefly derived). I have already referred to the effects of concentration, temperature and degree of dispersion. Other factors are solvate formation; electrolytic dissociation, in which solvate formation is probably involved; previous thermal treatment, as in the case of gelatine, which also shows an influence of mechanical treatment, even in the liquid state, in that its viscosity diminishes by repeated passage through a narrow tube and gives evidence of some kind of "structure"; inoculation with small quantities of a more viscous colloid, which produces a much greater effect than that due to its own viscosity; time, especially shown by the effect of the rate at which the temperature is changed; and finally the addition of electrolytes or non-electrolytes, which may raise or depress viscosity in the most varied manner. A particularly striking instance of large changes in viscosity produced by small changes in temperature is shown by such colloids as gelatines which form gels, and also by those which coagulate on heating. As an illustration we may take the change in the viscosity of a dilute albumin sol when heated (Fig. 68, from the paper by Wo. Ostwald). From 50° to 57° the viscosity decreases regularly. At 57°·5, just before the appearance of turbidity, a large increase occurs, which, at 60°, gives place to an equally steep decrease. After that, the curve forms practically a continuation of the direction of the first part below 57°, as if nothing had happened in the meantime.

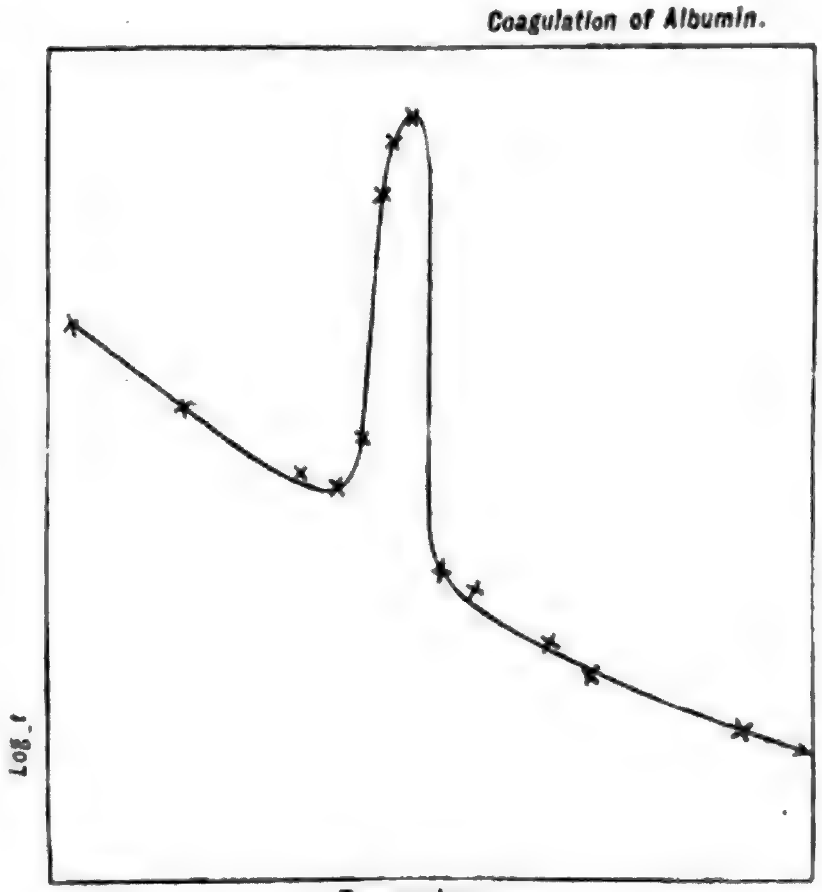


FIG. 68.—CHANGES IN VISCOSITY OF ALBUMIN IN THE PROCESS OF COAGULATION.

Abscissæ—temperature.
Ordinates—logarithms of the time of flow through the capillary tube of the viscosimeter.

(Wo. Ostwald, 1913.)

In the case of agar, the effect of concentration is very marked; from 0 to 1 per cent. the viscosity increases from that of water to several thousand times this value.

The general theory of the viscosity of such two-phase systems has been treated by Hatschek (1910-1913). Certain conclusions may be given here. Suppose the particles themselves are undeformable, then the viscosity is independent of their size and is a linear function of the volume of the dispersed phase only. The matter is more complicated in the case of two liquid phases, emulsions or emulsoid colloids, and the change of shape due to the shearing force must be taken into account. With emulsoids above a certain concentration, there is a very rapid rise of viscosity with further increase in concentration. The particular concentration at which this effect begins to show itself varies with different colloids and serves as a measure of their "lyophilic" properties or affinities for the solvent. With caseinogen it begins at 5 per cent., with glycogen at 25 per cent., with india-rubber at 0·4-0·5 per cent. The great swelling of india-rubber in its solvents, before the hydrosol is formed, is a familiar fact. It will be clear that, in the investigation of such systems, the rate of shear is an important factor,

since on this depends the degree to which the deformed droplets are able to return to their normal resting shape, spherical or polyhedral, according to the relative volume of the two phases. Hatschek (1913) has improved the apparatus of Couette, in which this rate of shear can be altered at will. It consists essentially of two concentric cylinders, the outer one of which can be rotated at a desired rate, while the inner one is suspended by a wire. The liquid is in the space between the two and the degree of torsion of the wire is measured by the deflection of a beam of light reflected from a mirror attached to the cylinder.

In this connection, some observations by Arisz (1913) are of interest. These experiments were made to determine the fluidity, that is, the inverse or reciprocal of viscosity, as a function of temperature in the case of the sol and gel of gelatine. It was found that a continuous curve is given, so that there is no break at any point and the process is a uniform one. The intensity of the Faraday effect and the elasticity were found to show similar continuity. The method used in the case of the gel was to determine the viscosity by the rate of change of shape under the action of a constant force.

SUMMARY

Water, of all substances known to us, is endowed with the most remarkable combination of properties, all of which play a part in contributing to the importance of its association with living processes.

Among these properties, we may note its high specific heat, its great latent heats of solidification and of vaporisation, its good conduction of heat, which is unusually high for a non-metal, its point of maximum density at 4° above its freezing point, its high surface tension, its transparency to radiant energy, its solvent powers, and, as a solvent, its chemical inertness is important, and its large dielectric constant. In the majority of these, it stands higher than any other substance, and where it is exceeded, it is only by one or two very exceptional liquids, such as ammonia and prussic acid. While some of these are dependent on each other, others appear to be independent. The manner in which each of these characteristics intervenes in relation to living organisms is given in the text.

Many of these properties find a satisfactory explanation in the nature of water as a polymerised compound, consisting of three degrees of aggregates—trihydrol, a compound of three molecules of H_2O, and apparently identical with ice; dihydrol, of two molecules, present in largest proportion in ordinary liquid water; and finally monohydrol, or steam, of single molecules. The relative proportion of these to one another changes as the temperature varies, so that passing upwards the concentration of the polymers decreases regularly.

The application of heat, therefore, has to do three things: decompose the polymers, heat the polymers and heat the single molecules; the anomalies connected with the specific heat of water are thus explained.

The point of maximum density at 4° can be explained on the assumption that ice, or trihydrol, exists in liquid water, since ice at 0° has a lower density than water at 0°; and, as water is heated from 0° upwards, there are two opposite processes going on, dilatation of the molecules, according to rule, and contraction, due to change of ice to water. Since the latter process is preponderant at the lower temperatures and nearly absent at the higher, there must be a point where the difference between them is least.

The unusual increase both of viscosity and of compressibility as the temperature falls is also explained by the existence of polymers.

Certain other properties of water are to be explained by the fact of its being, of all liquids, that one containing the greatest number of molecules per unit volume.

The proof of the existence of single molecules, monohydrol, in liquid water is given by consideration of solution volumes and will be found in the text.

Many solutes, both electrolytes, ions and non-electrolytes, take up a certain number of molecules of water, forming "hydrates." Whether this is to be regarded as chemical combination appears to be rather a matter of opinion.

It is shown that the theory of osmotic pressure, as given in Chapter VI., is not affected by the hydration of solutes nor by the polymerisation of solvent.

Water, to a very small extent, is electrolytically dissociated. The value of the dissociation constant, obtained by four independent methods, is practically identical, a satisfactory proof of the correctness of the assumption.

This electrolytic dissociation of water is the cause of the "hydrolytic dissociation" of salts of weak acids and bases dissolved therein.

The properties of water as a catalyst are, in many cases, of importance.

The concentration of water in reversible reactions of hydrolysis and synthesis is a potent factor in the regulation of reactions in protoplasmic systems. There are, no doubt, mechanisms of a colloidal nature present in such systems and effective in bringing about changes in the active concentration of water. Diminution of water favours synthesis, increase favours hydrolysis.

There is evidence that certain bacteria can be completely deprived of water without causing their death. When organisms become encysted, it appears that they do not become completely dried, but that the membrane of the cyst is practically impermeable to water.

When dry, both organisms and complex organic compounds, such as enzymes, can withstand, without destruction, a much higher temperature than in the presence of water.

The molecules of liquids in their movements past one another experience friction; this is known as their internal friction and gives rise to their viscosity.

The part played by the viscosity of the blood in causing the "peripheral resistance" of the arterial system is pointed out, and it is shown that this factor, on which depends, with a given heart beat, the height of the arterial pressure, is due to the internal friction of the blood and not to its friction against the walls of the blood vessels.

The viscosity of colloidal systems depends, in the main, on the degree of dispersion of the internal phase. In the case of suspensoids, the maximum of viscosity is at a medium degree of dispersion. In the case of emulsoids, where the internal phase is deformable, there are more factors to be taken into account, especially the rate of shear.

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CHAPTER IX

NUTRITION

THE USE OF FOOD

Food may be defined as any substance taken in by an organism and made use of for any purpose. The uses of food may be said to be threefold. When an organism is increasing in size, it is clear that the additional matter laid on must be obtained from without. In the adult organism, the main part of the food is used to afford energy for muscular movement, production of heat, etc. But there is also a small but essential part required for the repair of wear and tear on the part of the tissues themselves, even in the adult. This may be thought to be identical with growth, but we shall see later that there is evidence to show that certain things may be necessary for growth, although not so for maintenance. It is as if, after a machine has been constructed, certain working parts only require repair.

Perhaps an illustration may help to make these differences clear. A petrol motor in process of construction needs the supply of iron, steel, brass, copper, porcelain, insulation material, asbestos, and so on. Some of these cannot be replaced by any other; insulating material, for example, cannot be replaced by metal. We shall find analogous conditions in the growth of living things. When the engine is completed, fuel must be given in order that work may be done by it. This fuel does not enter as a constituent of the fabric, and corresponds to that part of our food which is utilised for the giving of energy. If the motor is kept at work, certain parts require replacement from time to time, owing to their wearing out: such are piston rings, linings of bearings, etc. These are the analogous parts to that fraction of our food which is needed for repair of tissue waste, or maintenance. We may note that certain parts practically never require renewal, such as the fly-wheel or the framework. The reader will probably ask, what does the lubricating oil represent? We must not, of course, expect to be able to push our simile to all details and it seems to fail here. The agents known as enzymes in some ways correspond to the lubricating oil, but these are formed by the organism itself. On the whole, water and salts are the nearest food constituents representing the function of the lubricant; they afford no energy, but are indispensable to the working of the living machine. We may also compare the waste products in the two cases. The products of combustion of petrol escape in the exhaust gases as carbon dioxide and water vapour, just as the same substances are given out in the gases expired from the lungs. The waste lubricating oil carries with it fine particles of metal, worn from the cylinder and bearings; in a similar way, the water excreted by the kidneys removes the products formed in the wear and tear of the tissues, in addition to other things, whose meaning will become plainer presently. For the present, attention may be called to our nitrogen food, the proteins, of which a part only is used for the giving of energy, the nitrogenous part being excreted as a waste product, urea, by the kidneys. We can imagine something of this kind in the case of the petrol motor; suppose that there were an incombustible impurity in the fuel, and that it were converted by the heat of the explosion into something very soluble in the lubricating oil of the cylinder, it would then pass out with the waste oil, which represents the urine.

NECESSARY CONSTITUENTS

From the three different purposes to which food is applied, it will be obvious that substances necessary for one object may not be so for another. There are, however, some food-stuffs which are indispensable for all purposes, such as oxygen, hydrogen, nitrogen, and carbon.

Oxygen.—This, although not commonly regarded as a food, is actually the most important of all. Life, except in rare cases of a special nature, is impossible without it for more than a very short time. As already pointed out (page 29) the energy of the animal body is derived from the oxidation of food. It is important to note that there is, in the animal, no formation of substances which endow the organism with more energy than that supplied to it in the food. Energy given out in one reaction may, however, be used to raise energy potential in another reaction, as we shall see exemplified in the case of muscle. In the green plant, on the contrary, energy derived from the sun is made use of to raise the energy of carbon dioxide and water to that of carbohydrate.

Water and Salts.—Although these substances afford no energy, their supply is essential for the numerous purposes made plain in the preceding chapters of this book. A continued supply is needed, since the kidney must excrete water in order to dissolve the waste products, and salts from the blood pass through the glomerular filter along with the water. Consideration of the osmotic pressure of these salts as they exist in the blood, about 3·5 atmospheres, shows that a large amount of work would be required to separate them from the water in which they are dissolved.

The value of *Carbon and Hydrogen* as giving energy by oxidation is obvious. Their heats of combustion are sufficient to show this. They are, of course, always in various forms of combination in food-stuffs, so that the whole of their energy is not available. It might appear that, for purposes of giving energy, hydrogen alone might serve, but it is unnecessary to state that it would be useless as gas and no chemical compounds except those with carbon are available. Similar remarks apply to carbon itself. These two elements are then always taken in combination and in fact partially oxidised, since the hydrocarbons are too inert chemically to admit of reaction under the conditions compatible with the existence of the protoplasmic system. The special value of carbon, with respect to the great variety of compounds which its peculiarities enable it to form, has been pointed out on page 41 above.

The position of *nitrogen* is somewhat different. As a direct source of energy its value is small. But there is, as we shall see later, a certain value in protein food, even as a source of *heat*, notwithstanding the fact that its nitrogen is almost immediately excreted unoxidised. It appears as if the amino-acids, produced by the action of enzymes on this protein food, after de-amination by the liver, leave certain residues which are, for some reason or other, more readily oxidised and utilised as sources of *heat*, perhaps because the two processes are parts of the same reaction, or, in other words, because of the "nascent" state of the ketonic or hydroxy-fatty acids formed.

It is clear that, for the growth or repair of structures containing nitrogen, this element must be supplied. The same may be said of *sulphur* and *phosphorus*, which are always found as constituents of cells.

Notwithstanding what has been said as to the value of nitrogen food, it is astonishing how little is absolutely necessary for the mere maintenance of life even in the higher animals. M'Collum (1911, 1, p. 212) found that pigs may be fed on a diet free from nitrogen for more than three weeks, without losing weight. Nitrogen is always excreted, none the less. In M'Collum's pigs, the nitrogen excreted per day amounted to 0·31 g. per pig of 84 lbs. weight. This then, in the case referred to, is the minimum amount which must be given, theoretically, if the loss of nitrogen is to be prevented. The amount of nitrogen given off from wear and tear is sometimes known as the endogenous protein metabolism. The value of 0·31 g. just given should be contrasted with that of 12 to 15 g. excreted on ordinary diet. In these particular animals, it appears that the minimum quantity required for repair is only about 2 per cent. of the whole protein metabolism on an ordinary diet. The question of the nitrogen minimum will come up for discussion subsequently.

Finally, it is obvious that products of secretion, containing particular elements,

require the supply of some food containing these elements. For example, the hydrochloric acid of the gastric juice must have chlorine. To form the hæmoglobin of the blood corpuscles, iron is necessary; since a number of these corpuscles are regularly broken up, new ones must be formed. Probably most of the iron required is obtained from the debris of the old cells, so that comparatively little further supply is needed.

To avoid misapprehension, it must be mentioned here that recent investigations have shown the necessity of minute quantities of certain organic substances, whose nature is as yet not understood. Details will be found below.

THE CHEMICAL COMPLEXITY OF THE FOOD-STUFFS REQUIRED

The *green plant* is able to obtain its carbon from the carbon dioxide of the air, its hydrogen from water, and its nitrogen from nitrates in the solutions bathing its roots. It is possible, therefore, to grow such plants as the bean, or better, the wallflower, from the seed to flowers and fruit, with its roots immersed in a solution containing merely potassium nitrate and some other inorganic salts, sulphates and phosphates of calcium. A trace of iron must be present. But this growth is only possible in the light and it is by the aid of radiant energy from the sun that the assimilation of carbon is made possible.

The methods by which instructive experiments of this kind can be performed will be found in the works of Darwin and Acton (1894, pp. 51-55) and of Macdougal (1901, pp. 223-232), in addition to many other textbooks of practical physiology of plants.

The green colouring matter, chlorophyll, by means of which the carbon assimilation of the green plant is effected, has been said to be the most interesting substance in existence, and, beyond doubt, the mechanism by which alone the higher animals themselves are enabled to maintain their life is of the utmost importance. The question will be discussed in Chapter XIX.

When we investigate the *fungi*, many of them highly organised plants, but devoid of chlorophyll, we find, as would be expected, that they cannot obtain their supply of carbon from carbon dioxide alone. Sugar appears to be their best source of carbon, but most carbon compounds, unless poisonous, suffice, with the exception of the very simplest ones, such as formic acid and urea.

What is perhaps more remarkable is that the *higher fungi* are unable even to use nitrates as a source of nitrogen, which the green plant is able to do. These higher fungi require ammonium salts, amines, or amino-acids; although urea cannot afford them carbon, it suffices as a source of nitrogen. Moulds and certain bacteria, lower fungi, are able to obtain nitrogen from nitrates, so that this capability is not entirely limited to the green plant, and it is not necessarily connected, as might be thought, with the use of the sun's energy for the assimilation of carbon. At the same time, we must remember that the obtaining of nitrogen from nitrates is common to all green plants, whereas it is only a few of the simplest fungi that possess it, and we find, moreover, especially amongst the bacteria, very specialised requirements as to the chemical nature of their food-stuffs. I may instance the fact that it was found impossible to cultivate the tubercle bacillus with success until glycerol was added to the medium. On the other hand, there are some bacteria which possess the very remarkable aptitude of using methane as a source of carbon (Söhngen, 1905). In this connection we may note that, although certain bacteria are able to utilise particular substances for food, it does not follow that this food is that on which they thrive best. In want of better, they can put up with it.

The *animal organism*, even in its lowest forms, the protozoa, is satisfied with nothing less complex than glucose as source of carbon. As regards nitrogen, the requirements appear to be different according to the purpose to which it is to be put, growth, maintenance, or source of energy. The experiments of Grafe (1912) appear to indicate that ammonium salts, in presence of excess of carbohydrate, may replace wear and tear in the dog and pig, although no tissue is laid on. Further facts bearing on this question will be found in the section on "Protein Metabolism" below, together with its probable explanation.

The *Protozoa* are apt to be considered as very primitive organisms, rudimentary ancestors of higher animals, because they are unicellular. But, although there is no doubt that higher animals have arisen in the course of evolution from simple creatures of this kind, one must admit that the protozoa, as we have them now, are complex, highly differentiated organisms. The Amœba, apparently, cannot be grown on a culture medium, unless it is supplied with bacteria, although dead ones suffice.

As a general rule, we may say that animals require food which has been previously built up by the plant. They feed either on vegetable matter or on other animals.

It was believed at one time that animals, at all events the higher ones, required nitrogen in the form of more or less complex proteins, but we have now definite proof that the products of hydrolysis of proteins, amino-acids, will prevent loss of nitrogen from the adult animal.

Optical Activity.—In connection with the remark made above as to the preference of one form of carbon or nitrogen food before another, it is interesting to note that, of the amino-acids, it is the *l*-series only which is utilised for the building up of the tissue proteins, although there is evidence that the opposite optical isomers can be used for energy purposes, although not so readily. In the case of carbohydrates, again, it is only the *d*-series that is easily utilised. The mistake is sometimes made, however, of stating this use of one series only as an absolute fact, whereas it is only relative. Pasteur (1860, p. 33 of the reprint in Ostwald's "Klassiker") in his classical work on the separation of the *d* and *l*-tartrates, used moulds to consume the dextro-acid and leave the other intact. As soon, however, as all the *d*-acid was exhausted, the mould proceeded to consume the *l*-acid, so that the rotatory power of the solution passed through a maximum. Other instances will be referred to when enzymes are under discussion, and the general question is treated in a later section of the present chapter. There is also preference for certain disaccharides, and here the utilisation is connected with the possession of particular enzymes which hydrolyse the disaccharide. The facts have been chiefly studied in the case of different species of yeasts. Emil Fischer has also shown (1884-1908) that, of all the possible carbohydrates of the general formula $C_nH_{2n}O_n$, ordinary yeasts can only act upon those in which the number of carbon atoms is three or a multiple of three; moreover, of those of the same constitution, but of different stereochemical configuration, a particular yeast will ferment one at a much greater rate than another.

One of the most striking examples is that of the sorbose bacterium, as studied by Bertrand (1896). Acting only on glycerol or on sugars with a terminal alcohol group (CH_2OH), it attacks a CHOH group near this one, transforming it into CO and thus producing a ketone. Moreover, the OH of the group attacked must not be next to the H of a neighbouring CHOH. Glycerol is thus oxidised into dihydroxyacetone.

Salts.—As already stated, these are necessary for all organisms, but the requirements as to particular salts vary considerably. This is one of the problems with which the agriculturist has to deal. Apart from nitrates, which do not come under the head of salts as such, potassium and calcium seem to be indispensable and other salts are more or less favourable. The reader is referred to the monograph by E. J. Russell (1912) for further information. As an illustration, we may refer to the pioneer work of Raulin (1870), some aspects of which have been mentioned above. By numerous experiments with different salts in different concentrations, it was found that for the growth of Aspergillus, a medium of the following composition gave better results than one in which any one of the constituents was omitted or present in another concentration :—

Water	1,500	Magnesium carbonate	0·40
Cane-sugar, cryst.	70	Ammonium sulphate	0·25
Tartaric acid	4	Zinc sulphate	0·07
Ammonium nitrate	4	Ferrous sulphate	0·07
Ammonium phosphate	0·60	Potassium silicate	0·07
Potassium carbonate	0·60		

Note that, while some of these substances are foods in the narrow sense of the

word, *i.e.*, utilised as actual constituents of the cells, or for energy or growth, such as sugar, ammonium nitrate, phosphate, iron, and potassium, others have additional functions. Tartaric acid keeps the solution acid and prevents the growth of bacteria; iron serves to neutralise, probably oxidise, injurious substances formed in the process of growth. The cane-sugar is first hydrolysed by an enzyme in the mould and lactose will not replace it, since the enzyme

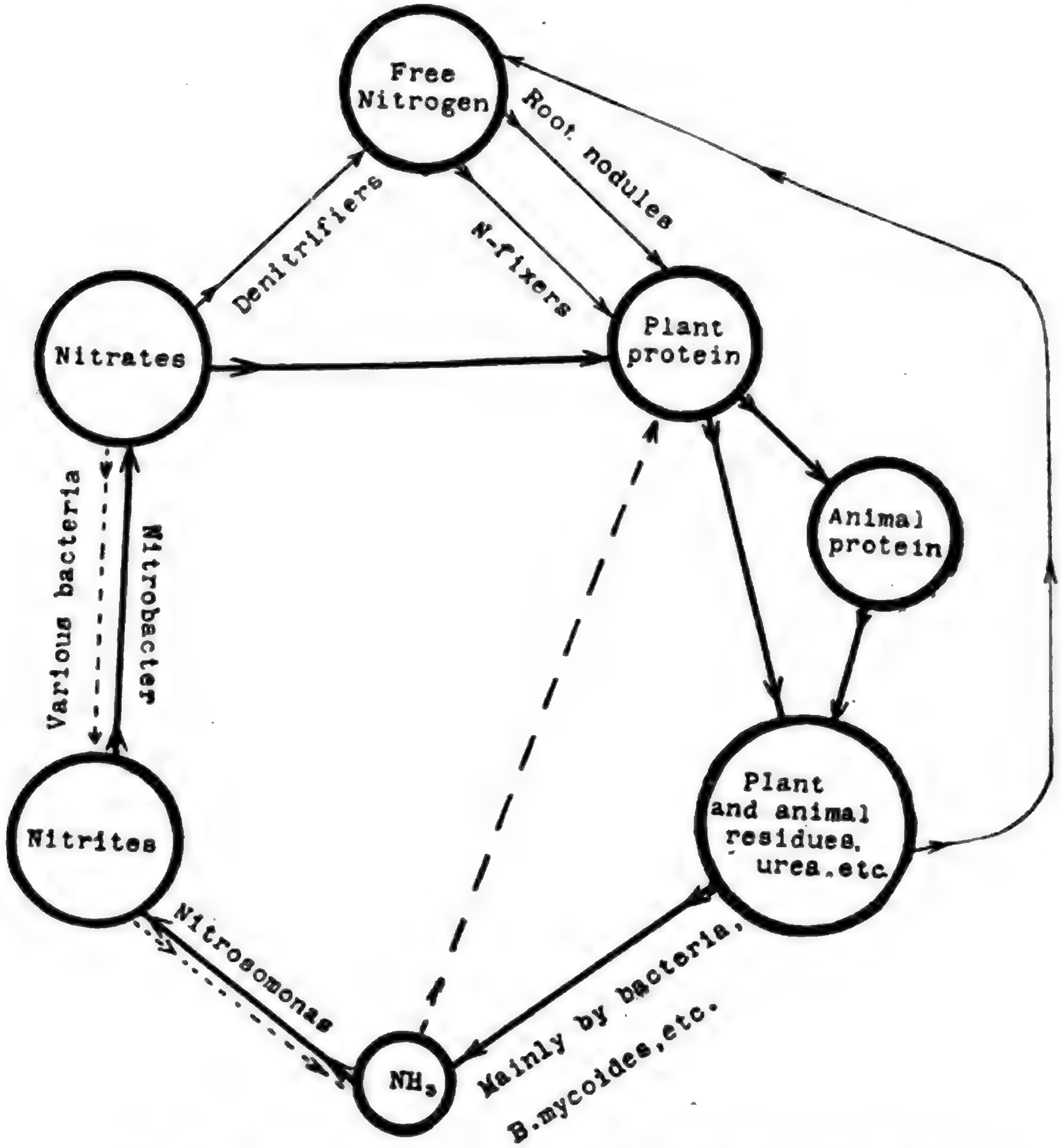


FIG. 69. DIAGRAM OF THE NITROGEN CYCLE, FROM THE ATMOSPHERE THROUGH PLANTS AND ANIMALS.

required to hydrolyse lactose is absent. Glucose, of course, can replace it, and it is of interest that alcohol, while retarding the germination of the spores, serves as an excellent source of carbon to the grown plant.

THE NITROGEN CYCLE

It will have become sufficiently obvious that the continued supply of carbon and hydrogen to living organisms in general is sufficiently provided for by the activity of the green plant in forming sugar from the carbon dioxide evolved in

combustion processes, including those of plants and animals, water being taken into the molecule in the process. Water and salts are also readily available and suffer no degradation of energy in passing through the organism. Nitrogen, on the other hand, must be presented to the green plant in the form of nitrate, in order that it may be further synthesised into a suitable form for the needs of the animal. Atmospheric nitrogen is useless for this purpose, and although ammonia, which is formed from animal excreta and from the debris of plants, chiefly by bacterial agency, can be utilised by the higher plant as a source of nitrogen, it is by no means the normal and efficient one (see Russell, 1912, pp. 30-31); moreover, in the conversion of the residues from plant and animal into ammonia, a certain amount is always lost in the form of free nitrogen. It is therefore a matter of fundamental importance for the continued existence of life on the earth that some means should be present for the conversion of nitrogen gas into a form available for the growth of the green plant, and also that an effective mechanism should exist for the transformation of ammonia into nitrates.

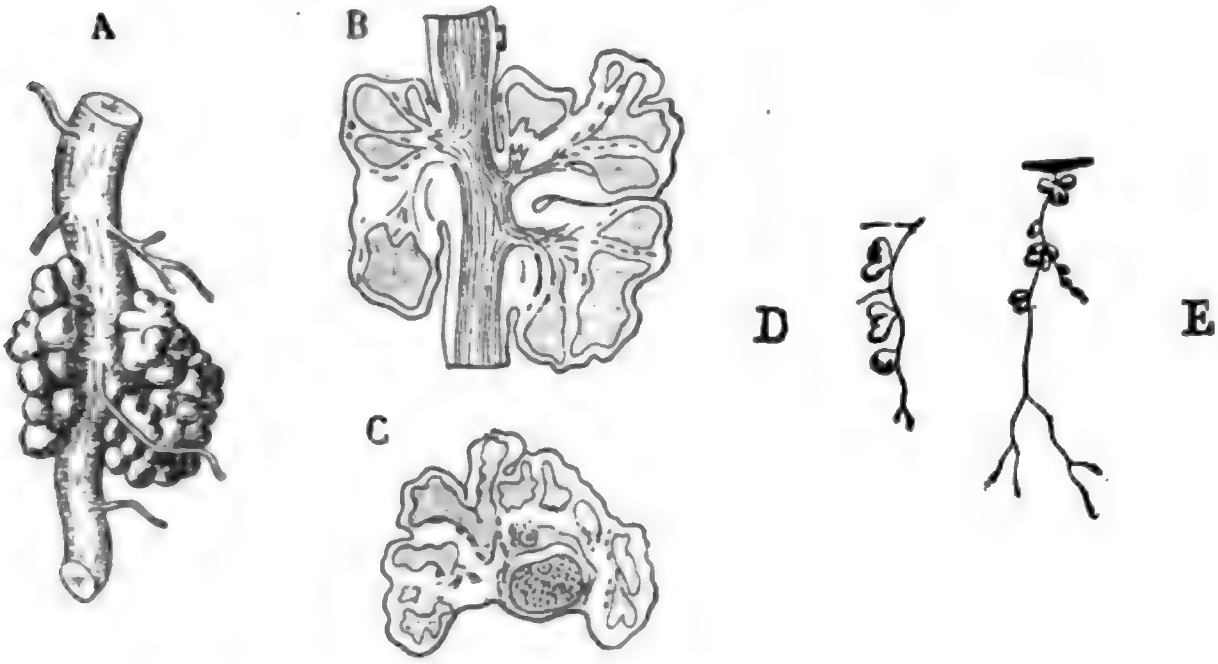


FIG. 70. ROOT TUBERCLES OF LEGUMINOSÆ.—About natural size.

A, Lupin. External view.
B, Diagram of longitudinal section of lupin tubercle.
C, Diagram of transverse section.
D, Cracca minor.
E, Clover.

(*A*, *B*, *C* from Woronine; *D* and *E* from Vuillemin. *See* Lutz, 1904, pp. 70 and 71.)

FIG. 71. MICRO-ORGANISMS FROM TUBERCLE OF DESMODIUM GYRANS.

Pure culture. Stained with methylene blue. Size of organisms, 1·3 $\mu \times$ 3·7 μ.

(Miss Dawson, 1900, Fig. 6.)

The diagram given in Fig. 69 will serve to elucidate the process by which these requirements are met and will enable a shorter verbal account to be given than would otherwise be necessary. Additional details may be found in the monograph by Russell (1912, chap. iv.).

Following the direction of the arrows in the figure, and starting from atmospheric nitrogen, we notice that there are two ways in which this is "fixed" in a form available for the use of plants. In the first place, there are bacteria in the soil which are able to obtain their nitrogen from the atmosphere. Their existence was clearly shown by Vinogradsky (1895). The chief forms are a *Clostridium*, anaerobic, isolated by the observer named, and *Azotobacter*, aerobic, discovered by Beijerinck (1901).

Vinogradsky recognised that the process required energy to be supplied, since it is endothermic; in experimental work, glucose is added, and a considerable amount is consumed: each milligram of nitrogen fixed requiring the oxidation of 500 mg. of sugar. In the soil, decomposition products of cellulose apparently take the place of the glucose as sources of energy.

The second process is peculiar to the leguminous plants, together with a few others. Russell points out (1912, p. 84, footnote) that it was known to the Romans that the growth of vetches (a leguminous plant) on ground afterwards used for wheat caused an increased crop of this latter. In Vergil's "Georgics," Book I., lines 73 and following, the farmer is recommended, "before sowing his yellow wheat, to take off a crop of beans, with their rattling pods, or of the frail offspring of the vetch, or of lupins, with their brittle stalks and rustling straw." All of these are leguminous plants, be it noted.

The word translated "straw" in this passage is "silva"; but it is difficult to see in what sense a field of lupins could be called a "wood."

The reason of the beneficial effect of such plants was discovered by Hellriegel and Wilfarth (1888). They showed, in the first place, that, adding together the nitrogen of the soil in a particular culture pot to that of the plants grown in it, there is, in the case of oats, always a little less than that originally present, but, in the case of peas, always *more*. This could only come from the nitrogen of the atmosphere. At this time it was already known that the nodules on the roots of leguminous plants contain bacteria, and the hypothesis was a natural one that these organisms were able to fix nitrogen and hand it over to the plant in some way. Beijerinck (1888) isolated the organisms from the root nodules, but, although they must be present in the soil, since extracts of soil on which leguminous plants have been grown will infect the roots of other plants of the same order, it was found impossible to discover them therein. After entry into the root hairs, they multiply rapidly and presently form a nodule on a part of the root. Inside the nodules they change to Y-shaped "bacterioids." Fig. 70 shows roots with nodules and Fig. 71 (from the paper by Miss Dawson, 1900) gives the appearance of the bacteroids. The chemistry of the process is unknown. The final product is supposed to be soluble protein, which is passed on to the plant. From what we know as to the nitrogen supply to the tissues in animals, it seems more likely that it is an amino-acid or amide. In any case, the facts are of great practical importance, since leguminosæ are among the commonest plants, and the process is independent of organic matter in the soil. The carbohydrate required to afford energy for the work of the micro-organisms is obtained from the plant on which it grows. The growth of these plants, then, always leads to increase of organic nitrogen in the soil.

Owing to the two processes named, the green plant has been enabled to form proteins. If eaten by an animal, these proteins serve as nitrogen food for it. The waste products containing nitrogen, from both animal and plant, some of them of simple composition, such as urea, others more or less insoluble solids, on return to the soil, are converted into ammonium salts, mainly by the agency of bacteria, although it is said that the process may take place slowly in the presence of antiseptics. The reaction probably consists, in the case of the more complex compounds, in the production of amino-acids and subsequent hydrolysis or oxidation of these. During the process, however, a considerable loss of nitrogen in the gaseous form occurs, as presented by the thin line in the diagram. This loss is supposed to be due to oxidising bacteria, but the question is not yet decided.

The ammonium salts thus formed are capable of serving to a certain extent as nitrogen food for the green plant, indicated by the interrupted line in the diagram leading back to plant proteins; but they are not efficient in this respect and, according to Russell (1912, p. 31), plants fed only on ammonium salts as source of nitrogen, really suffer from nitrogen starvation.

A means of converting ammonia into nitrates is clearly an essential requirement. This is actually provided in the following way. The first step is the formation of ammonium carbonate, by simple chemical reaction with alkaline carbonates, so far as not already present in this form. This ammonium carbonate is rapidly

converted by a special organism, *Nitrosomonas*, into nitrite and this nitrite again into nitrate by another organism, *Nitrobacter*. These bacteria are always present in normal soil and act with such rapidity that only traces of either ammonia or nitrite can be detected in the soil.

The fact can readily be observed by adding about half a gram of soil to 50 c.c. of a culture fluid of the following composition :—

Ammonium sulphate	0·5	Magnesium sulphate	0·1
Sodium chloride	0·5	Ferrous sulphate	0·1
Potassium acid phosphate	0·25	Water to	1,000

and adding to 50 c.c. about half a gram of solid magnesium carbonate to preserve neutrality. After four weeks or so, the ammonia will be found to have disappeared and nitrate to have taken its place. The existence of the latter can be shown by the reaction with diphenylamine sulphate in sulphuric acid.

The chemical process occurring is unknown, but the bacteria concerned have rather extraordinary properties. Carbon dioxide will serve as source of carbon, and in fact it seems that, in cultures *in vitro*, other more complex carbon compounds are injurious, especially glucose or peptone. But, in order to synthesise cell stuffs from carbon dioxide, a supply of energy from without is necessary. Light is out of the question, since the organisms do not possess chlorophyll and, in point of fact, light is actually fatal to them. It has been suggested that the oxidation of ammonia and of nitrite might afford sufficient energy. The injurious effect of organic matter only applies to artificial cultures; in the soil, glucose has a beneficial effect, although other sugars are inert and nitrogen compounds injurious. The organisms are killed by a temperature of 45° C. or by the absence of oxygen.

Preparations containing nitrifying bacteria and called "nitragin" have been sold for the purpose of improving the soil, but their beneficial effect is doubtful (see Miss Dawson's investigations, 1898 and 1900). There is evidence that deficiency of these organisms may be due to excessive numbers of protozoa, which consume them (Russell, 1912, p. 118).

The normal use of the nitrates is to form plant proteins, but, in the absence of oxygen, they rapidly disappear if not made use of. They are converted back to nitrates and ammonia on the one hand, as shown by the dotted lines on the diagram, and to free nitrogen, on the other hand, as shown by the thin line. Many various forms of bacteria are concerned in the process, producing also a number of substances other than those named.

On account of the importance of nitrates as food for plants, and indirectly for animals, any means of obtaining them from the atmosphere by non-living agency is of value. It has long been known that the oxygen and nitrogen of the air, in presence of water vapour, can be caused to combine by the electric spark, forming nitric acid. Of recent years, the process has been developed on a commercial scale by the use of large electric arcs, spread out by magnetic action; considerable quantities of nitric acid are made yearly in situations where there is abundant water power. In Birkeland's process, worked chiefly in Norway, the arc is produced by an electromotive force of 5,000 volts and is spread out into a disc of 2 m. in diameter, having a temperature of some 3,000°. Again, Calcium cyanamide is formed when nitrogen is passed over calcium carbide, heated to 1,000°. Calcium carbide itself is produced in the electric furnace from lime and carbon. Calcium cyanamide gives ammonia when acted on by water and was, at one time, advocated for the purpose of supplying nitrogen to plants. Another process, apparently of great efficiency, has recently been discovered by Haber (1914). By compressing a mixture of hydrogen and nitrogen and causing it to be acted on by a certain catalyst at a high temperature, ammonia is formed by direct combination, in large proportion. By passing this over another catalyst, it is oxidised to nitric acid.

THE THREE CLASSES OF ORGANIC FOOD-STUFFS

For the food of the higher animals, it is usually of advantage to make up the requirements by a combination of certain proportions of fat, carbohydrate, and protein. Fat is of value on account of its high potential energy, being less

oxidised than carbohydrate. It is not, of course, a necessary article of diet, since it can be formed in the organism from carbohydrates. A certain amount of carbohydrate appears to be a matter of necessity, as we shall see later. And, although the whole of the energy requirements could be supplied by protein, it would be very wasteful, since only a comparatively small amount of nitrogen is actually required. The point is that a minimum total energy value, usually expressed in heat units, must be supplied to an animal, in order to prevent loss of body substance.

SPECIAL REQUIREMENTS

As organisms increase in complexity in the course of evolution, it appears that their capacity of synthesising the innumerable compounds of which they consist

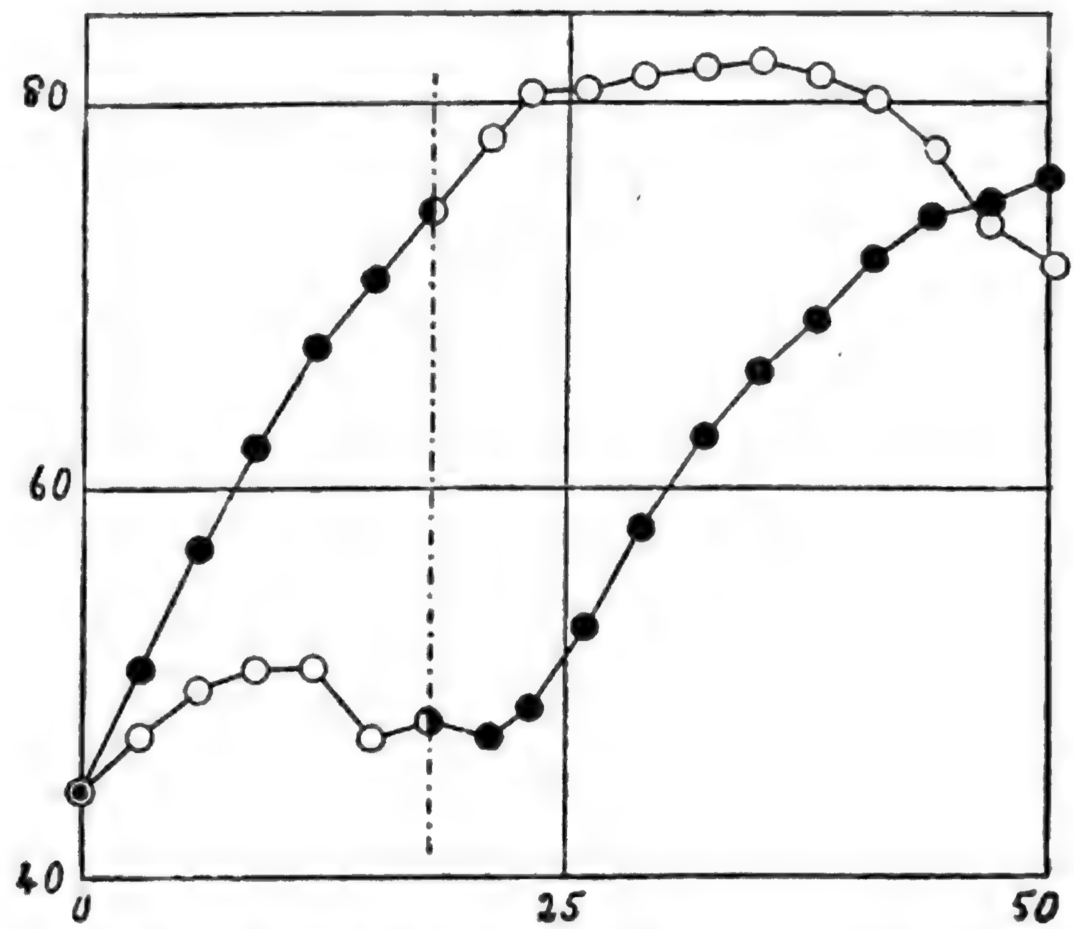


FIG. 72. FAILURE OF RATS TO GROW ON A PURE SYNTHETIC DIET.

Ordinates—average weight of animal in grams.
Abscissæ—time in days.
Lower curve (as far as the 18th day)—eight male rats on pure diet, free from "accessory factor."
Upper curve—eight similar rats taking, in addition, 3 c.c. of milk per day.
On the 18th day, marked by vertical dotted line, the addition of milk was changed from one set to the other.

(Hopkins, 1912, p. 433.)

is diminished. There are certain differences as regards requirements for growth, maintenance, or energy, so that a diet which is adequate as a supply of energy, that is, one which has a sufficient calorie value, may be inadequate for replacing wear and tear, while a diet which is adequate for this purpose may be unable to allow growth to take place. Hopkins (1912) showed, for example, that young rats if fed on mixtures of pure caseinogen, fat, carbohydrate, and salts rapidly ceased to grow, although the energy value of the diet was amply sufficient for the purpose. On the other hand, if a minute quantity of fresh milk (3 c.c. per day) was added to the diet, growth recommenced and went on rapidly. Fig. 72 is a representation of one of these experiments. It is to be noted that these amounts of milk merely added some 4 per cent. or less to the total solid eaten and are altogether inadequate to account for the increased growth, which amounted to about half a gram per rat per day, whereas the total solid content of the milk

added would not be more than about 0·08 g. The experiment is referred to at this point merely as an illustration and will be discussed further later on. We may note, however, that the active constituent of milk is not one of the known ones. Milk freed from protein and salts is equally effective and it was shown some time ago by Lunin (1880, p. 37) that a "synthetic" milk, containing all the known constituents, will not serve as a complete diet.

We will now proceed to attempt some kind of analysis of the different sorts of those special constituents of food, of which only a minute amount is required, but which is essential. At the outset, it is clear that such substances cannot be required for energy purposes directly, so that the part they play must be either in growth or maintenance of cells, or else as hormones or catalysts, the function of which will be explained presently. As regards growth and maintenance, there is evidence that a particular substance may be necessary for the former but not so for the latter.

1. *As Components of Tissues*

The tissue proteins, as we have seen (page 103), are composed of a considerable number of different amino-acids, so that this question resolves itself into the capability of the organism to synthesise for itself all these constituents, or whether it has to depend upon the supply of some of them from the outside. The green plant needs no further nitrogenous food than nitrates, so that the constituents of its proteins, which are identical with those of animal protein, must be formed in the organism itself. One of these plant proteins, gliadin from wheat, contains alanine, valine, leucine, phenylalanine, tyrosine, serine, cystine, proline, aspartic and glutamic acids, tryptophane, arginine and histidine. For the chemical constitution of these, the reader is referred to the monographs by Plimmer (1912, 1913).

An interesting direct proof of synthesis of some of them has been given by Abderhalden and Rona (1905), who grew the mould, Aspergillus, on a culture fluid containing only potassium nitrate as source of nitrogen and cane-sugar as source of carbon. The amino-acids were then separated by the esterification method of Emil Fischer (see Plimmer's monograph, 1912, pp. 22, etc.). Glycine, alanine, leucine, aspartic and glutamic acids were separated and identified. The absence of aromatic derivatives is notable.

Although the plant is capable of such varied synthetic processes, it is remarkable that the power has been lost to a large extent by the animal organism. A certain limited capacity is, however, known to exist, and it is possible that further instances may be found in the future. Glycine can be formed in the higher organism, as was shown by Magnus-Levy (1907).

Two lines of evidence may be cited. The proteins of milk contain only about 0·3 per cent. of glycine, but a sucking calf can build up 78 g. of tissue protein out of 100 g. of milk. Now this animal tissue protein contains at least 2·5 g. of glycine. Again, when benzoic acid is given to an animal, it becomes conjugated with glycine to form hippuric acid (benzoyl-glycine), which is excreted by the kidney. A rabbit which was estimated to contain 6·6 g. of glycine, excreted 8 g. of this amino-acid in combination with benzoic acid, when the latter was administered to it. This experiment is, perhaps, not altogether convincing, on account of the uncertainty in the actual content of the rabbit in glycine, although it is improbable that all the glycine-containing tissues should be decomposed in such a way as to give up the whole of their glycine.

Hart and Humphrey (1915) showed that special proteins are necessary for the best production of milk; hence the capacity of cows to synthesize amino-acids is limited.

We must admit the possibility of the formation of alanine also in the following way. Embden and Kraus (1912) showed that lactic acid is formed by the liver from glycogen, and Knoop (1910) that the liver can synthesise α-hydroxy-acids with ammonia to form the corresponding α-amino-acids; from lactic acid, alanine is therefore obtained thus:—

$$\begin{array}{c} CH_3 \\ | \\ CHOH \\ | \\ COOH \end{array} + NH_3 = \begin{array}{c} CH_3 \\ | \\ CH.NH_2 \\ | \\ COOH \end{array} + H_2O$$

Moreover, Embden and Schmitz (1910) actually found that a liver rich in glycogen showed a considerable formation of alanine when ammonium chloride was added to the perfusion fluid. The liver can also use the ketonic acid for synthesis of the

corresponding amino-acid. It is only necessary then to supply this organ with the appropriate hydroxy or ketonic acid in order that an amino-acid may be formed by it. However, we know as yet of no α-hydroxy or α-ketonic acid produced by the organism with the exception of lactic and pyruvic acids, both of which give alanine. Dakin and Dudley (1913, 1) have shown that many amino-acids, in fact all examined by them, namely glycine, alanine, valine, leucine, phenyl-alanine and aspartic acid, undergo spontaneous dissociation at low temperatures into the corresponding α-ketonic aldehyde and ammonia. The reaction is no doubt reversible and probably catalysed by an enzyme, so that the formation of an amino-acid from the hydroxy-acid seems to pass through the intermediate stage of the corresponding ketonic aldehyde. **Thus**:—

$$R.CH.NH_2.COOH \rightleftarrows R.CO.CHO + NH_3.$$

Glycine, alanine, aspartic and glutamic acids, and histidine have been shown to form glucose in phloridzin diabetes (see Ringer and Lusk, 1910). Since the reactions concerned are reversible, these amino-acids might be formed in the organism from glucose and ammonia.

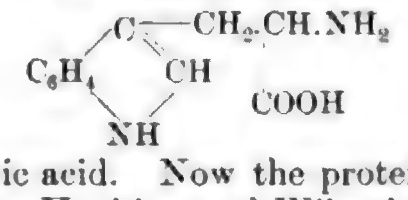
The experiments of Henriques and Hansen (1904) are of interest in this connection. They found that rats could be maintained in nitrogen equilibrium, that is, without loss of nitrogen, if they were fed on the mono-amino-fraction of the products of digestion of proteins. Since the tissue proteins contain diamino-acids, there must have been synthesis of these, if the results are correct.

Hindhede (1914) finds that the minimum nitrogen required with a diet of bread is the same as that with potatoes; it seems that we must conclude that no special kind of amino-acids is necessary, at all events for maintenance.

On the other hand, we have to remember that it is possible for a diet to contain all that is necessary for repair of adult tissue, although it may not contain some constituent required for new growth. If this constituent cannot be formed in the organism itself, it is clear that the diet will be inadequate for growth. This fact has already been insisted upon. Osborne and Mendel (1912, 2) have maintained adult rats for as long as 530 days on gliadin and protein-free milk. Now gliadin is wanting in glycine and lysine, which are necessary as constituents for the growth of new tissues, and, in fact, experiment showed that the diet in question was actually inadequate for growth. It seems probable that the wear and tear of the cells does not involve disintegration of that particular protein which contains lysine, or that the group containing lysine may be left intact when other parts are split off.

There is another way in which the want of a particular constituent in diet may be of importance. As we shall see in more detail later, there are numerous substances produced by various organs which act upon other organs, such as adrenaline. These are essential for the normal functions of the organism, so that if they require for their production some particular chemical grouping, which the organism itself is unable to supply, this grouping must be present in the food, otherwise normal life is impossible.

There is no doubt that *Tryptophane* is of especial importance. This substance is found in most proteins used for food, and has the constitution:—



that is, indol-amino-propionic acid. Now the protein of maize, zein, is peculiar in containing no tryptophane. Hopkins and Wilcock (1906) found that mice were unable to live for more than about twenty days on a diet of zein, carbohydrate, and fat. Whereas, if tryptophane were added, the animals not only lived much longer, but were obviously in better condition.

Further evidence is afforded by the experiments of Henriques (1907) in conjunction with those of Abderhalden and Frank (1910). It has been mentioned above that the products of complete enzymic hydrolysis of proteins are sufficient as protein diet. Further, if the proteins are hydrolysed by the action of acid, provided that the hydrolysis be only carried on for six hours, it was found by Henriques that the products were also able to maintain nitrogen equilibrium

whereas if the hydrolysis were allowed to proceed for seventeen hours, the resulting product was useless. The only detectable difference was that the tryptophane reaction was still present after six hours, absent after seventeen hours. Abderhalden and Frank completely hydrolysed horse flesh by boiling with sulphuric acid and then added 0·5 per cent. of trytophane. The mixture was adequate for dogs. It is impossible to say definitely what the function of tryptophane is, perhaps for the elaboration of some internal secretion, as suggested by Hopkins.

The experiments of Osborne, Mendel, and Ferry (1912, 1) prove undoubtedly that a protein as unlike the tissue proteins as gliadin is, can serve for the purpose of forming new tissues, through the intervention of the adult animal. The experiments given on pp. 485 and 486 of their paper are instructive. A rat was fed for 178 days on a diet consisting of gliadin only as protein, along with protein-free milk carbohydrate, and fat; at the end of this time, four young rats were born. Of these, after thirty days' feeding by the mother's milk, three were put on a normal diet while the fourth received gliadin food similar to that which the mother had been receiving all the time. The three former grew well, but the latter quickly began to fail. The diet which sufficed for the adult, not only for its own maintenance, but also for production of young and secretion of normal milk, was insufficient for the growth of the young animal. It will be noticed that the requisite substances were present in the milk secreted by the mother, since, while fed on this, the young rats showed normal growth. The adult, therefore, has the power of forming some substances out of others in the diet, which power is not possessed by the young animal, so that they must be supplied to it from without. It will be seen that these experiments do not altogether decide the question as to whether the failure to grow was due to want of lysine in the diet or to want of some "accessory factor." The fact that when casein was substituted for gliadin, the diet was adequate, indicates that the former was the case.

The function of zinc in the growth of Aspergillus should also be referred to under the head of accessory food-stuffs, since it appears from the work of Bertrand and Javillier (1912, 2) that it enters as a constituent into the plant itself. Of course, it is not necessarily implied that it forms a part of the chemical structure of protoplasm itself.

2. *As Accessory Factors.*—We have seen that gliadin, in the experiments of Osborne and Mendel, although lacking in lysine and insufficient for growth, appears to be adequate for maintenance, although the results of Hopkins and Neville (1913) throw some doubt on its adequacy for any length of time even for this purpose. Now there are other substances, of which a very minute amount only is required, but in whose absence a diet, otherwise perfectly adequate even for growth, is unable to preserve life. There are several sets of facts derived from different kinds of phenomena which prove this statement.

As many of these experiments were made on rats and mice, and some investigators hold that these small animals are inappropriate for metabolism experiments, it is well to refer to the circumstances pointed out by Hopkins (1912, p. 427) that for the kind of experiments in question such small animals are especially valuable; a number can be dealt with at the same time, and the fact that their metabolic processes are rapid is important, especially for experiments on growth.

To turn to the experiments themselves, Hopkins (1912), one of whose curves is given in Fig. 72 (page 254), showed that a trace of fresh milk added to a diet, on which otherwise rats ceased to grow and ultimately died in about twenty-seven days, made it perfectly normal. We notice that even maintenance at the weight attained, except for a short time, is impossible without the addition. The active substance in milk must have been present in extraordinarily minute amount. Protein-free alcoholic extracts of milk solids, as well as the ether extract of the dry residue of the alcoholic extract, containing no inorganic constituents, as also the boiled watery extract of mangolds, were effective. The constituent is therefore neither protein nor salts; lactose can also be excluded, since the addition of it to the diet is useless, and we have seen from Lunin's experiments that a diet containing casein, butter, lactose, and salts is insufficient as a diet.

We may make a rough calculation as to the maximum possible amount of the active constituent in 2 c.c. of milk thus: milk contains only about 4 per cent. of ether-soluble constituents, so that 2 c.c. would contain 0·08 g. Moreover, Stepp's experiments (1909 and 1911) enable us to exclude the greater part of these. This observer fed mice with bread and milk which had been extracted with alcohol and ether; he found that such a diet kept them alive for only three to four weeks, whereas if a minute amount of the dry residue from the extracts were added, they lived indefinitely and appeared to be in normal condition. In further work (1913) Stepp finds that ether alone does not extract the important constituent from dry food, although alcohol alone does so. He also found that the addition of the neutral fat of milk was ineffective; as were also lecithin, cholesterol, cephalin, cerebron, or phytin. We may therefore subtract from the above 0·08 g. in Hopkins' diet, that part consisting of fat, lecithin, cholesterol, etc. This would leave scarcely more than a few milligrams, if so much. Other evidence is that boiled watery extracts of mangolds, which would not contain more than a trace of lipoids, are active, as Hopkins found. We must therefore agree with Hopkins that a catalytic or stimulating influence of some kind is more likely than that an actual tissue constituent is supplied.

Stepp holds that a "lipoid" of some kind is responsible for the result produced by the extracts in question. There are, however, other substances present in bread and milk which are soluble in alcohol and even to some extent in ether.

Osborne, Mendel, Ferry, and Wakeman (1913) find that the "accessory factor" for growth, contained in milk, goes into the fatty part of the butter. This contains neither nitrogen nor phosphorus, so that the active substance is not a lecithin-like substance, nor like Funk's "vitamines," to be mentioned presently. The fact that it is soluble in fat does not, of course, necessitate the conclusion that it is of fatty nature. Funk's vitamine must belong to another class of substances, or, more probably, the nitrogenous substance which he has analysed is not the really active one.

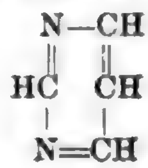
According to M'Collum and Davis (1915), there are two groups of these factors, neither of which is effective alone. Rats will not grow on polished rice, even if wheat germ be added; nor if butter alone be added. Both together make the diet adequate. That contained in wheat germ is soluble in water, not in fat, and may be called "water-soluble B factor"; the other is the "fat-soluble A factor." In Hopkins' experiments, fresh milk contained both. The deleterious effects due to the absence of these factors do not appear at once. They are subject to a delay of some days. This shows that they do not rapidly disappear, although they must be present in very small amount. The fact indicates that they do not undergo chemical change, like food-stuffs properly so called. The way in which they act is unknown at present, but the chemist will be reminded of the phenomena called "catalytic," to be discussed in Chapter X. They appear to be excreted unchanged in the urine (Gaglio, 1919). Researches on scurvy show that a third factor, the anti-scorbutic or C-factor, must be added (Harden and Zilwa, 1919).

There is no reason to suppose that the diet, containing amino-acids as sole source of nitrogen, which was found by Loewi and others to be capable of maintenance, or even a certain degree of laying on of protein, was free from the "accessory factors" in question, since unextracted carbohydrate was added to the food.

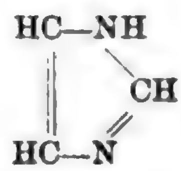
There is, on the whole, considerable evidence that a diet, wanting in something necessary for *growth*, may nevertheless be capable of *keeping up* the general condition in a normal manner. An interesting and, if confirmed, practically important application of this fact is reported by Sweet, Corson-White, and Saxon (1913 and 1915). Since tumours, such as cancer, are growths, it seemed that it should be possible to retard their increase by a diet insufficient for growth, while adequate for maintenance. The experiments showed that mice, fed on well-washed gluten from wheat, together with starch, lard, lactose, and salts, could be kept at a constant weight, that is, without growth, for thirty days or more. Comparing animals on this diet, inoculated with a rapidly growing tumour at the same time as those on normal diet, a considerable difference was found in the rate of growth of the tumour, which was much slower in those on the restricted diet. One mouse, for example, on restricted diet, at fifty-two days after inoculation, had at this time a scarcely visible tumour of 4 mm. in diameter. The mouse was then put on normal diet of bread, corn, etc., and, thirty days later, the tumour was nearly as big as the mouse itself. Prof. Hopkins informs me that he had already, before 1913, obtained similar results.

We may next consider shortly the results obtained by various observers, with respect to certain *diseases produced by the absence* of some substances of a similar

kind to those which we have discussed in the preceding pages. It has been clearly shown by Fraser and Stanton (1911) that the disease known as "*Beri-Beri*" is due to the exclusive feeding on rice which has had the pericarp and most of the underlying layer removed by "polishing." Fowls fed on such rice develop a polyneuritis (inflammation and subsequent degeneration of peripheral nerves), which is similar to that occurring in beri-beri, and can therefore be used to study the disease experimentally. If the polishings be added to the white rice, the animals remain healthy and those suffering from the disorder can be cured. As to the nature of the protective substances whose absence entails the disease, it was found that heating to 120° for two hours destroys them. They are soluble in slightly acid 91 per cent. alcohol. Further evidence on the nature of these substances has been brought forward by Funk (1911, 1912), who has separated a substance from rice polishings, and also from yeast, milk, ox-brain, and lime-juice, which, in very small doses, 0·02 to 0·04 g. by the mouth, cures polyneuritis in fowls. Funk states further that it has the properties of a pyrimidine base. Pyrimidine is:—



and is, together with iminazol:—



the characteristic constituent of the nucleins. Marked improvement was, in fact, obtained by giving certain substances related to the nucleins, such as thymus, nucleic acid, guanosin, etc. Funk suggests the name "vitamines" for the class. They are remarkably active; a quantity containing only 0·4 mg. of nitrogen cures a pigeon. An interesting point is that pigeons severely affected with polyneuritis recovered in six to twelve hours, and frequently seemed quite well after three hours. It appears that the paralysis can have been due to a functional disturbance only of the axis cylinders, although the medullary sheath was degenerated. It is impossible that recovery of degenerated axons could take place in so short a time. It appears that a normal medullary sheath is not absolutely necessary for nerve conduction. It should be mentioned that the constitution assigned by Funk to these "vitamines" is not generally accepted. We have seen above (page 257) that the active substance in butter contains no nitrogen, so that it seems probable that the substance investigated by Funk was not the active one, and that this latter was present only in traces. Indeed, Funk himself appears to have come to the conclusion later that this is the case, although he holds that the active substance is of basic nature, and precipitated by phospho-tungstic acid. The possibility has not yet been excluded, however, that the precipitate might carry down the active material by adsorption.

Scurvy has also been shown by Holst and Frölich (1912) to be due to a deficiency of some essential constituent in preserved foods, but present in all kinds of fresh material, animal or vegetable. It will be remembered how Captain Cook, in his second voyage, was successful in avoiding this disease, although the voyage lasted 1,000 days. He says (1776, p. 405): "We came to few places where either the art of man or nature did not afford some sort of refreshment or other, either of the animal or vegetable kind. It was my first care to procure what could be met with of either by every means in my power, and to oblige our people to make use thereof, both by my example and authority; but the benefits arising from such refreshments soon became so obvious that I had little occasion to employ either the one or the other."

The extreme sensitiveness to the want of some particular substance in small amount does not seem to be limited to the higher animals. Wildiers (1901) stated that if a normal artificial culture medium is inoculated with yeast in too small an amount, there is no growth, whereas, if the same quantity is added to sterilised

beer wort, the growth is vigorous. The effect of adding larger quantities is said to be due to the presence of a substance which Wildiers calls "*bios*," provisionally, until its chemical nature is known. This bios is found in all cultures of growing yeast. If the culture medium is inoculated with an insufficient quantity of yeast, and, at the same time, with small quantities of boiled yeast extract, even if filtered through porous clay, growth is ensured. The beneficial effect of phosphate on fermentation by yeast is well known, so that it is necessary to note that the artificial culture fluid used contained this salt already. A culture medium may sometimes become infected with yeast from the air, and it seems difficult to understand how bios could be taken with it; however, according to the observations of Kossovics (1896), it seems possible that bacteria might produce some substance of this nature. Since this work seems to have been somewhat overlooked, it is advisable to give some further details. It is interesting to note that the work was done at the University of Louvain, whose tragic fate has aroused the indignation of the whole civilised world.

As to the chemical nature of bios, we may note that it is soluble in 80 per cent. alcohol, not in absolute alcohol nor in ether. It is not precipitated by lead acetate, phospho-molybdic nor phospho-tungstic acid, nor by mercuric chloride. It is present in Liebig's meat extract and commercial peptone as well as in decoction of germinated malt (beer wort) before the action of yeast. It is not contained in the products of peptic or tryptic digestion of pure proteins. Thymus nucleic acid does not give the result. Whatever it may be, it is evidently not the same substance as that of Hopkins, since it is insoluble in ether, nor is it Funk's "vitamine" since it is not thrown down by precipitants of organic bases. An important point is that it is not produced by the yeast plant itself in the course of its growth, in fact it seems to disappear. If the culture to which originally a certain amount of bios was added be boiled and concentrated, it is found necessary to add at least that amount of the concentrated extract which would contain the original quantity of bios in order to ensure growth in a new experiment. Wildiers calls attention to the circumstance that the results seem to give a possible explanation of the famous contest between Liebig and Pasteur. The latter inoculated his cultures with a fragment of yeast of the size of the head of a *large* pin. This was probably about the lowest limit of "bios," so that, if Liebig took a smaller amount, he would not get growth. As Wildiers remarks (p. 328), if Liebig had accepted Pasteur's invitation to see his experiments, the discrepancy would probably have been explained. We see also how minute is the amount of bios required. A smaller quantity than the optimal will allow of a very slow growth, but, under the microscope, there are to be seen, at one time, very few living cells, and it appears that, in order that a new cell may grow, it has to wait for the death of an old one in order to obtain the bios required. On the whole there appears to be some essential structural unit which the yeast cell is unable to form for itself.

Abel Amand (1903) answers some possible objections to these experiments. We saw that the influence of the number of cells was eliminated in that the *same* number was ineffective in Pasteur's medium, effective in malt extract. Amand's experiments were made to test the suggestion that the bios added was effective by counteracting some poisonous substance in the water used for the culture fluid, some "oligodynamic action" as described above (page 22?). Copper was suggested. The water from glass stills, however, gave the same results. The chemicals used were also tested, and, although the details must be read in the original paper, the possibility of poisonous constituents seems satisfactorily excluded. Moreover, one may recall the experiments of Ringer, in which the poisonous action of distilled water was abolished by calcium, which is a constituent of the yeast culture medium. Amand, in a further paper (1904), shows that bios disappears and cannot be extracted from the cells, so that it is either used for synthesis of a more complex substance or undergoes spontaneous decomposition. According to Devloo (1906) the active principle is a base, precipitated by mercuric chloride in presence of barium hydroxide. It can be made to replace choline, at all events partially, in lecithin. It may naturally occur in the form of the base of a lecithin-like substance. It is not choline itself nor can it be prepared from choline. See also the papers by Williams (1919) and by Backman (1919).

Twort and Ingram (1912) have made the interesting observation that *Jöhne's bacillus*, responsible for the pseudo-tubercular enteritis of oxen, will only grow on a medium to which dead tubercle bacilli, or some other "acid-fast" bacilli, have been added. The essential substance is extracted by alcohol.

The work of Bottomley (1914) is also of interest. He finds that substances which stimulate plant growth are formed in sphagnum peat when it is incubated with cultures of certain aerobic soil bacteria. They are very active; the watery extract of 0·18 g. of peat thus treated caused the growth of Primula seedlings in six weeks to be double that of control plants. They seem to be allied to the "accessory factors" of diet.

Thornton and Smith (1914) find that the chlorophyll-containing protozoon,

Euglena, is unable to grow and multiply in a pure saline medium, although all the elements supposed to be necessary are present. If, however, a trace of tyrosine be added, growth is vigorous. It seems that the tyrosine does not serve as a food-stuff, since the active amount is so small (0·4 mgm. in 10 c.c.). Moreover, no growth occurs in the dark, so that the organism obtains its food from the carbon dioxide of the air and the mineral substances of the culture fluid. The tyrosine disappears, it is true, but this is due to the action of bacteria, unavoidably present in small numbers. See also E. J. Allen (1914) on the growth of diatoms.

The manganese and zinc required for the normal growth of *Aspergillus* may also be mentioned in the present connection.

Dorothy Jordan Lloyd (1916) points out that the results she obtained with meningococcus suggest that stimulating factors act as catalysts, increasing the rate of protein metabolism. Failure of growth without them may be due, not to absence of reaction, but to the fact that its rate is too slow.

Emil Fischer

Fig. 73. Portrait of Emil Fischer.

There is one possibility that has not been referred to as yet. We saw in Chapter V. how important a part the cell membrane plays in vital phenomena. Now, the actual amount of material contained in these membranes is almost infinitesimal, yet it might well be that there is something absolutely necessary to its proper constitution, a substance which may be gradually lost in the process of activity. One thinks of Clark's experiments (1913, 2) on the effect of prolonged perfusion with a simple saline solution, which washes away something which can be replaced by an ether extract of the dry residue of an alcoholic extract of dry serum (see page 211 above). The properties of lecithin may occur to the reader, but this, as well as cholesterol, seems to be excluded by the experiments of Stepp, who found neither of these, nor indeed other known "lipoids," capable of replacing the active substance in milk.

An important conclusion with respect to the general theory of nutrition is to be drawn from the various facts given in the present section. As was pointed out by Starling and myself (1906, p. 696) and by Hopkins (1906, p. 395), independently, it is not sufficient to estimate the value of a diet, as to whether it is an adequate one or not, merely by its calorie value or its content in fat, carbohydrate, and protein; the presence or absence of the small quantity of the "accessory factors" or "hormones" must also be taken into account.

THE METABOLISM OF PROTEINS

Our next task is to discuss briefly the changes undergone by the three classes

of organic food-stuffs as they pass through the organism, commencing our study with proteins.

The Constitution of Proteins.—As was first definitely and completely shown by Emil Fischer (1899-1906), proteins, animal and vegetable, are composed of a series of amino-acids united by elimination of water, as described on page 103 above. This work is of such fundamental importance that I have thought it necessary to introduce a portrait of Fischer in Fig. 73.

The following constituents have been isolated from various proteins:—

Mono-amino-mono-carboxylic Acids.

Glycine (amino-acetic), alanine (amino-propionic), amino-butyric, valine (amino-isovalerianic), leucine (amino-isobutyl-acetic), isoleucine (α-amino-β-methyl-β-ethyl-propionic), phenyl-alanine, tyrosine (para-oxyphenyl-amino-propionic), serine (α-amino-β-oxy-propionic), cystine (condensation of 2 molecules of α-amino-β-thio-propionic acid, the sulphur constituent of proteins).

Mono-amino-di-carboxylic Acids.

Aspartic (α-amino-succinic), glutamic (α-amino-glutaric).

Di-amino-mono-carboxylic Acids.

Arginine (α-amino-δ-guanido-valerianic acid). Guanidine is

$$\begin{array}{c} NH = C - NH_2. \\ \quad\;\; | \\ \quad\;\; NH_2 \end{array}$$

Lysine (di-amino-caproic) that is:—

$$NH_2 - CH_2 - CH_2 - CH_2 - CH_2 - CH(NH_2) - COOH.$$

Heterocyclic Compounds.

Histidine (β-iminazol-α-amino-propionic acid).

Proline (α-pyrrolidine carboxylic acid). Pyrrolidine is:—

$$\begin{array}{ccc} CH_2 & - & CH_2 \\ | & & | \\ CH_2 & & CH_2 \\ & \diagdown\;\diagup & \\ & NH & \end{array}$$

Oxy-proline (γ- or β-oxy-α-pyrrolidine carboxylic acid).

Tryptophane (constitution given on page 256).

And also ammonia. Note that the amino-acids are all of the α type, in which the NH_2 is next to the carboxyl group.

For further details see the monographs by Plimmer (1912, 1913).

How far have all the constituents been accounted for? The analysis of zein by Osborne and his co-workers account for 85·4 per cent. of the total nitrogen of the protein and, considering the inevitable losses in the mono-carboxylic acids, the result must be regarded as very satisfactory.

These amino-acids combine together in the way already indicated, that is:—

$$-CO\,\boxed{OH \quad H}\,NH-$$

becomes, by elimination of water:—

$$-CO-NH-$$

which is known as the "*peptide linkage*."

In this way, Fischer has prepared a large number of peptides, containing two or more amino-acids, in great variety.

The proteins of the tissues may, therefore, be regarded as made up by various selection out of the list given above, and in different relative proportion. Thus, while gelatine contains 16 per cent. of glycine and 0·9 per cent. of glutamic acid, gliadin of wheat contains no glycine, or extremely little, and 43 per cent. of glutamic acid.

We see that there are very few *free* NH_2 groups in a protein.

Van Slyke and Birchard (1914), in fact, show that the only free NH_2 groups in all the native proteins examined, a considerable number, amount to one-half of those of the lysine contained therein. All the other amino-groups are condensed into peptide linkings.

Methods of estimating the amount of nitrogen combined in the form of NH_2 are of value in determining approximately whether a given protein is a mixture of several simpler ones or is one single large molecule. We have also a means of following the degree of hydrolysis in the course of digestion or under the action of acid. There are two methods available for this purpose.

Sörensen's method depends on the fact that NH_2 groups react with formaldehyde to form methylene-imino groups :—

$$RNH_2 + CHOH = RNCH_2 + H_2O.$$

When proteins are acted on by formaldehyde, the basic groups are eliminated and the free carboxyl can be titrated with acid in the usual way. This fact was shown by Schiff (see Plimmer's monograph, 1912, 1913).

Van Slyke's method depends on the fact that primary amino-groups react with nitrous acid thus :—

$$RNH_2 + HNO_2 = ROH + H_2O + N_2.$$

The nitrogen gas evolved is measured (van Slyke, 1912 and 1913).

By these methods we find that proteins do not contain more than one per cent. of their nitrogen in the NH_2 form.

There are several classes of proteins, distinguished by their different properties, in addition to those conjugated with other substances, such as nucleins or carbohydrates. The *nomenclature* of these substances has been agreed upon by the Chemical and Physiological Societies of England and America and should be always used. It will be found in Plimmer's monograph (1912, pp. 1 and 2).

We have next to inquire what becomes of the proteins taken as food before the end products of their metabolism are excreted. Schwann (1839, p. 229) (p. 193 of Sydenham Society's translation) introduced the name "metabolic" to express the chemical changes which the constituents of the body undergo. He speaks of "phenomena which are related to chemical changes, both of the constituents of the cell itself and of the surrounding material; these may be called metabolic phenomena (τὸ μεταβολικὸν, that which is inclined to produce or suffer change)" ("Diese kann man metabolische Erscheinungen nennen, τὸ μεταβολικὸν, was Unwandlung hervorzubringen oder zu erleiden geneigt ist").

In the space that is available in such a book as the present one, it is impossible to describe in detail all the numerous facts which are known as to these phenomena, important as they are. For the subject of protein metabolism, the reader should consult the monograph by Cathcart (1912).

There is strong evidence to show that the proteins of the food are completely hydrolysed into amino-acids, before being absorbed. For some time, however, it was held that re-synthesis to proteins took place in the wall of the intestine. This view was due to the fact that it had not been possible to detect amino-acids, nor even peptones, in the blood. It seems, on *à priori* grounds alone, that such an immediate resynthesis would be a very inappropriate one. Suppose that a particular tissue protein is to be built up and that this protein, while containing glycine, contains very little glutamic acid, also that another cell protein contains no glycine, but a large amount of glutamic acid. Now, if the synthetic protein supplied by the blood contained the right proportion of amino-acids for the one, a large quantity of it would have to be taken up by the other, in order to satisfy its requirements, and the remaining part of it, containing the excess of the particular amino-acid not wanted, would be wasted. Even if this last were utilised in some other way, it seems a useless process for a protein to be synthesised in the wall of the intestine, merely to be broken up again when it reaches the cells.

Direct evidence, moreover, is not wanting at the present time showing that amino-acids do actually exist in the blood. According to van Slyke and Meyer (1912), in the first place, the blood of a dog which has received no food for twenty-four hours contains amino-acids equivalent to 4 mg. of nitrogen per 100 c.c. If meat is given, the value rises to 10 mg. during digestion.

The reason why the quantity is so small is that these amino-acids are rapidly taken up by the tissues in some form, as we shall see later; it was found, for example, that if 12 g. of alanine were injected into a vein of a dog during ten minutes, only 1·5 g. remained in the blood five minutes later, although only 1·5 g. had been excreted by the kidney.

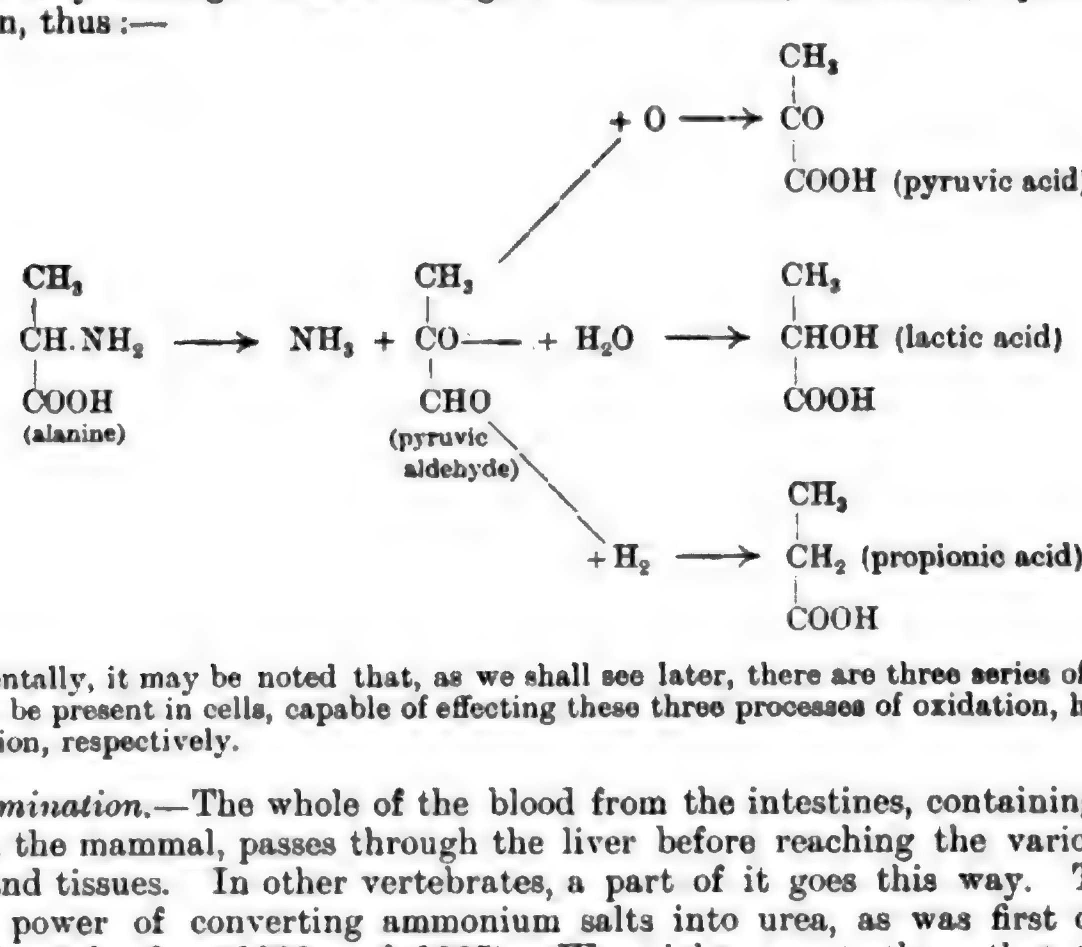
Folin and Denis (1912) came to the same conclusions and could find no evidence whatever of protein synthesis in the intestine. Abel (1913), again, by the ingenious method of dialysis of the living blood, referred to above (page 83), has been able to collect as much as 20 g. of amino-acids from the blood of three or four dogs, so that it is possible to separate them and find out which are present. The fact that they have now been detected in the blood is due to the improved methods devised for their determination, and we may conclude that they do actually, as such, reach the tissue cells. Delaunay (1913) showed their presence in the body-fluids of invertebrates. Confirmatory evidence is afforded by the experiments of Buglia (1912, p. 184), who found that sufficient nitrogen food to meet requirements can be injected, in the form of amino-acids, into the veins slowly without disturbance.

We have already seen evidence that the actual amount of nitrogen required for repair is very small, and further details will be given later. How, then, is the remaining nitrogen of a considerable protein diet dealt with? If we start from the other end, as it were, we find by experiment that the amino-acid nitrogen not needed for growth or repair appears in the urine as urea, while the rest of the molecule is ultimately converted into carbon dioxide and water.

From the chemical standpoint, the most obvious stages between an amino-acid and urea are, first, de-amination, by which ammonia is split off and some derivative of a fatty acid formed; and, secondly, the ammonia is converted into urea, while the hydrocarbon acid is burnt up for the purpose of affording energy.

This view is, in fact, a part of the theory of *protein metabolism* associated with the name of *Folin* (1905). We have to inquire what evidence there is of such reactions occurring in the living organism, and where they occur. A similar theory was propounded by Delaunay (1913, 1).

With regard to the first step in the process, it has been shown by Dakin and Dudley (1913, 2) that an α-amino-acid in solution in water undergoes spontaneous dissociation into the corresponding α-ketonic aldehyde and ammonia and this fact makes it probable that the process is accelerated in the organism by an enzyme. Alanine becomes in this way pyruvic aldehyde and ammonia. The ketonic aldehyde may undergo further change in three modes, oxidation, hydrolysis, or reduction, thus:—



Incidentally, it may be noted that, as we shall see later, there are three series of enzymes, known to be present in cells, capable of effecting these three processes of oxidation, hydrolysis, or reduction, respectively.

De-amination.—The whole of the blood from the intestines, containing amino-acids, in the mammal, passes through the liver before reaching the various other organs and tissues. In other vertebrates, a part of it goes this way. The liver has the power of converting ammonium salts into urea, as was first definitely proved by Schröder (1882 and 1885). We might expect, then, that the main body of the amino-acids would be first de-aminated in the liver, the resulting ammonia converted to urea, while the fatty acid remainders would be sent on to

the tissues. Part of the amino-acids must be supposed to escape the action of the liver, to afford the nitrogen required for growth and maintenance.

The "Specific Dynamic Action" of Rubner.—Although there is very little energy lost in de-amination (Leathes, 1906, p. 154), amino-acids appear to have some special advantage in the production of heat. If we measure the heat produced after a known amount of carbohydrate or fat has been eaten, the extra amount is equal to that taken in. If protein is taken, *more* heat is given out by some stimulation of the cells. The observations of Anderson and Lusk (1917) show that protein has no special value as regards conversion to work. The amount of energy value in food required by a dog to do a particular piece of work was the same whether derived from its own store or from a diet of carbohydrate or of protein, namely, 0·58 kilogrammetre per kilogram of body weight transported one metre. *But* when protein was used, the extra amount of energy due to its specific dynamic action was given off *in addition* to that utilised as work. In other words, when the work was done by the expenditure of protein, the body lost more energy than when the same work was done at the expense of carbohydrate. It is possible that in exceptional exposure to cold, the extra heat given off under protein diet may be of temporary advantage, but under ordinary conditions it is difficult to regard it as other than waste. On the whole, the "specific dynamic action" appears to be an incidental occurrence in the mode of utilisation of protein, of doubtful physiological value.

Lusk (1915) holds that "specific dynamic action" is due to an action exerted on the cells by amino-acids, so that their metabolism is increased. The stimulus is not the amino-acids themselves, but probably the hydroxy-acids resulting from their de-amination.

Again, certain experiments tend to show that the liver has not much more power of de-amination than the other cells of the organism. The experiments of Lang (1904) and of Miss Bostock (1911) have shown that tissues *in vitro* are capable of de-aminating amino-acids to some extent, but that the process is not quite the same as that in the living organism, since amides are more readily acted on *in vitro* than are amino-acids, while the contrary is the case in the organism. That the main part of the amino-acids absorbed by the intestine escape immediate de-amination by the liver is also shown by the results of van Slyke and Meyer (1912, p. 408). The blood of the femoral artery contained in one experiment, before feeding, 3·7 mg. of amino-acid nitrogen per 100 c.c., and after feeding, 8·6 mg., while that of the portal vein, at the same time, contained very little more, namely, 9·5 mg. This means that there was a loss of only 0·9 mg. in traversing the liver, not more than would be expected if the liver only de-aminated as much in proportion to its size as other organs. But the reaction may be slow.

After large doses of amino-acids the de-aminated products can be detected in the urine. Lactic acid from alanine (Neuberg and Langstein, 1903), glyceric acid from diamino-propionic acid (Mayer, 1904) may be referred to.

Moulds, bacteria, yeast, and the larvæ of flies have also been shown to split off ammonia from amino-acids.

It seems probable that amino-acids are supplied to the tissues, and, with the exception of that small part used for repair or growth, are de-aminated there.

The fatty acid part is utilised for supply of energy and the next question is the fate of the ammonia.

The great activity of the liver in the conversion of ammonia to urea makes it probable that the main part of the ammonia from the tissues is converted into urea in this organ. When an Eck's fistula is made, that is, when a connection is made between the portal vein and the vena cava, so that the liver is practically cut out of the circulation, there is a great increase of ammonia in the blood. Normally, also, there is much more ammonia in the portal blood going to the liver than in that of the hepatic veins coming from it. The investigations of Nencki and of Salaskin may be consulted (see Cathcart's monograph, 1912, p. 51).

On the other hand, Folin and Denis (1912) hold that the tissues themselves have the power of converting ammonia into urea. They find that the urea content of the blood of the hepatic vein, after the injection into the lumen of the intestine of various proteins and amino-acids, is not greater than that of

the femoral artery. This implies that the liver has not added any more urea to the blood than was already in that arriving to it from the other parts of the animal. Should this be so, all tissues must take part in the formation of urea. In the words of these authors (1912, p. 161), "the food protein reaches the tissues in the form of amino-acids, and those amino-acids which are not needed for the rebuilding of broken-down body material are not rebuilt either into protein or protoplasm, but are broken down and their nitrogen converted into urea."

The results of van Slyke and Meyer (1913, 2), contrary to those of Folin and Denis, are in favour of the first view, that the liver is the chief, if not the only, situation where de-amination occurs. Amino-acids, as we saw, are taken up rapidly by all tissues. Those taken up by the liver disappear again in a comparatively short time, but, during the time required for this disappearance from the liver, no appreciable diminution has occurred in that stored in the muscles. From other organs also they disappear less rapidly than they do from the liver. This diminution of amino-acid content of the liver is accompanied by increase of urea in the blood. The liver, therefore, continually tends to decrease the amino-acid content of the blood, and, since there is always an equilibrium between the amino-acid concentration in the blood and that in the tissues, as the liver removes these acids more passes from other tissues to restore equilibrium. We see then that it is not necessary that de-amination should be performed by any tissue other than the liver. In a further paper (1913, 3) the same investigators show that in starvation the amino-acid content of the tissues does not decrease. It is most probably renewed by autolysis of protein, so that the amino-acid protein system appears to be that of a reversible chemical reaction. This view is supported by the fact that feeding with large quantities of protein does not increase the amino-acids of the tissues, so that any nitrogen stored beyond the normal amount of amino-acid must apparently be in the form of protein.

In any case, we may conclude that nothing is left of the old discussion between Pflüger and Voit as to the necessity of food protein becoming living protoplasm before being utilised.

If the view taken be correct, we see also that, so long as the "accessory factors" are present, there is no necessity for the food proteins to be of similar constitution to the tissues. In fact, experimental evidence confirms this deduction; except for differences in degree of digestibility, and so on, there does not seem to be any particular preference for one protein rather than another.

A further consequence is that feeding with pure amino-acids should be possible. The evidence that this can be done has been referred to above (page 256).

As to the *chemical mechanism of de-amination* we have little information. Probably all the three reactions given above (page 264) are concerned.

An old observation by Strecker referred to by Bach (1913, 1, pp. 157, 158) is interesting as a possibility of the formation of hydroxy-acids and aldehydes from amino-acids. Alloxan reacts in stages with amino-acids thus:—

$$\mathrm{OC}\left\langle\begin{array}{l}\mathrm{NH-CO}\\ \mathrm{NH-CO}\end{array}\right\rangle\mathrm{CO}+\mathrm{R.CH}\left\langle\begin{array}{l}\mathrm{NH_2}\\ \mathrm{COOH}\end{array}\right.+3\mathrm{H.OH}=$$

$$\mathrm{OC}\left\langle\begin{array}{l}\mathrm{NH-CO}\\ \mathrm{NH-CO}\end{array}\right\rangle\mathrm{CH.OH}+\mathrm{R.CH}\left\langle\begin{array}{l}\mathrm{OH}\\ \mathrm{OH}\end{array}\right.+\mathrm{CO}\left\langle\begin{array}{l}\mathrm{OH}\\ \mathrm{OH}\end{array}\right.+\mathrm{NH_3}=$$

$$\mathrm{OC}\left\langle\begin{array}{l}\mathrm{NH-CO}\\ \mathrm{NH-CO}\end{array}\right\rangle\mathrm{CH.NH_2}+\mathrm{R.CHO}+\mathrm{CO_2}+3\mathrm{H_2O}.$$

The general facts of protein metabolism may be looked at from a slightly different point of view, as in the original expression of his theory by Folin (1905), who carried out a large number of analyses of urine on two kinds of diets, rich and poor in nitrogen, but both practically free from purines, creatine or creatinine (see later, page 270). Comparing the two series, we note that there are some products of metabolism which maintain a nearly constant figure, while others are much greater under rich nitrogen food than under food poor in nitrogen. The constant products

are chiefly creatinine and neutral sulphur; to a less extent, uric acid and ethereal sulphates. The variable products are urea and inorganic sulphates, *not* creatinine and probably not neutral sulphur. The former obviously represent some constant form of metabolism always proceeding, to which Folin gave the name of tissue or *endogenous* metabolism, and to the variable type, that is, to the proteins used for de-amination and giving of energy, the name intermediate or *exogenous* metabolism. This view has been generally accepted, although some minor points as to the complete distinction of the particular products in each case have been taken exception to. Folin himself admits that urea is probably an end product of both. According to Cathcart (1912, p. 95), the output of creatinine is itself subject to small changes when the protein ingested is altered.

The practical interest and importance of the question rests on the fact that the amount of nitrogen which it is absolutely necessary to take in the food, is, in theory, limited to that required to form new tissues or replace wear and tear; that is, the endogenous fraction. Now the nitrogenous food is the most costly part of a diet, so that it is of some importance to know how far it can wisely be reduced. The value of the *nitrogen minimum* is therefore a question requiring discussion.

Firstly, what is the excretion of nitrogen in *starvation*? This may be taken as the index of the waste of tissues, with certain qualifications. But it will be clear that, for our present purpose, what we want to know is the loss of nitrogen when sufficient carbohydrate is supplied for energy purposes, nitrogen being absent from the food. The tissue proteins begin to break down in complete starvation in order to afford the energy demanded by certain organs of vital necessity, such as the heart, so that the issue becomes confused. If a particular excretory product, under normal diet, were definitely known to be a product of endogenous metabolism and of this alone, it would be more satisfactory to determine the amount of this substance excreted. We cannot, as yet, be quite certain as to the existence of such a product, although, according to Cathcart (1909), creatine, a constituent of muscle tissue, is such a product, present only in starvation, so that the study of its excretion gives valuable information, to which reference will be made later. Some doubts, however, have been thrown by Graham and Poulton (1913) on the cogency of the method used to estimate creatine in the urine. According to these workers, there is no satisfactory evidence of the presence of this substance in the urine, under any circumstances. According to Cathcart and Orr (1914), however, these results do not affect the conclusions drawn by Cathcart from his experiments. But, in any case, there is, according to M'Collum (1911, 1), another index in the output of creatinine, a product obtained from creatine by removal of water (see below, page 270). This is a constant fraction of the total nitrogen eliminated after a long-continued diet free from nitrogen. In the pig, the creatinine nitrogen is, under these conditions, 18·5 per cent. of the total nitrogen excreted. So that if the creatinine nitrogen be multiplied by 5·5, the total nitrogen resulting from endogenous metabolism is obtained. This conclusion rests on the fact that creatine or creatinine is a characteristic product of the breakdown of muscular tissue.

In a later paper M'Collum and Hoagland (1913) show that this conclusion requires certain modifications, which must be taken into account in attempts to make use of it. There are, they say, at least two types of endogenous protein metabolism, one which can be stimulated to increased production of ammonia by feeding with mineral acids, or to hippuric acid production by glycine, while the other, which is represented by creatinine, remains unaffected by these agents.

From Cathcart's experiments (1909) it appears that, in man, the total output of nitrogen on a carbohydrate diet, free from nitrogen, is about 5 g. per day. Now Voit had laid it down that the daily intake of protein should be 120 g., equivalent to 18 g. of nitrogen. Chittenden (1905) regards this as far too much and was able to maintain nitrogen equilibrium on 6 g. of nitrogen (40 g. of protein) in various classes of men engaged in different kinds of work. There is no doubt that Voit's amount is considerably in excess of that taken by a large number of men. For example, Hamill and Schryver (1906) determined

the nitrogen output in the urine of seven of us who were working in the Physiological Laboratory of University College, London, at that time. No alteration was made in our occupations nor in the food taken, except that a dinner of the Physiological Society occurred on one day, which tended to increase the general average. The values obtained were from 0·16 to 0·2 g. of nitrogen per kilogram of body weight, or an average of 13·5 g. per individual, equivalent to 93 g. of protein; a value only three-quarters of that given by Voit, although rather more than twice that regarded by Chittenden as adequate.

A point of interest is that in Rowntree's "Poverty; a Study in Town Life," the author has adopted Atwater's standard of 125 g. as the minimum protein and consequently finds that 27 per cent. of the population of York are living in poverty, because their protein consumption is below this figure. In point of fact, the lowest value found was 89 g., very little below that of the laboratory workers, and this applied only to those whose weekly wage was below twenty-six shillings. Caution must then be exercised in drawing conclusions as to social conditions from protein consumption. One would have to conclude that physiologists as a class are living in poverty.

Cathcart (1912, p. 69) regards 90 g. of protein as an average value, from his own experience. This author's discussion of the question will be found on pp. 66 to 72 of his monograph (1912). We may note that Sivén (1901) found it possible to maintain nitrogen equilibrium on 4·52 g. of nitrogen (=28·3 g. of protein) per day. But there seems some evidence that continued existence on so low a protein diet may entail low resistance to external influences, such as infection, although this effect is by no means clearly made out and the results of Hindhede, to be given immediately, show that it is not necessarily the case.

The degree of activity of the organism is naturally to be taken into account. We may recall M'Collum's experiments on pigs (1911), in which the total nitrogen required for maintenance appears to be only 2·6 g. for a pig of about the weight of a man.

The recent work of Hindhede (1913) affords some valuable data on the question before us. In his experiments, care was taken that the total calorie value of the food was abundant, a point of essential importance, as Cathcart points out (1912, p. 70), and not sufficiently ensured in some of the experiments of Chittenden, in which it was too low. A further point of importance in Hindhede's experiments is that they were continued for a considerable time. A strong, healthy young man of 70 kg. weight, a laboratory servant in the Nutrition Institute of Copenhagen, was the chief subject. It was found that, while continuing to perform all his usual duties, he was able to live on a diet consisting only of potatoes, apparently new potatoes, together with margarine and a little onion for flavour, and containing, on the average, only 4·425 g. of nitrogen per day. This experiment lasted 178 days and although 75 g. of nitrogen had actually been lost from the body, it was not possible to discover that the subject was otherwise in any different condition than at the beginning of the period. From the figures given, it appears that he was in nitrogen equilibrium during the actual time on which this diet was taken, and that the loss of nitrogen occurred in one or two short periods in which less nitrogen was taken, owing to replacement of the greater part of the potatoes by fruit. During the 150 days on which the potato diet was taken, nitrogen equilibrium was present on 5 g. of nitrogen per day. Taking one particular period of nineteen days, in which all the conditions were especially satisfactory, nitrogen equilibrium was maintained on only 3·5 g. per day. It is to be remembered that, on this potato diet, which seems to be the only one which can be put up with for so long a time, it was impossible to reduce the nitrogen further without diminishing the calorie value below that which was found to be essential, namely 4,000 calories per day. We may remark also that the method of cooking the food was found to be a matter of great importance, so that it should be sufficiently palatable to be taken with relish in large enough quantities to give the calorie value required, in fact, about 2·2 to 3·5 kg., according to the severity of the work done. To assign proper value to the experiments, it is pointed out that the subject was really more than an ordinary laboratory servant; he performed the duties of an assistant, working fourteen to sixteen hours a day, extremely active and taking great interest, not only in the experiments described, but also in the work as a whole. We may note the high calorie value of the diet; that given by Voit for soldiers in war-time had an energy value of only 3,575

calories, although it contained 145 g. of protein, equivalent to 23·2 g. of nitrogen.

A second experimental period was undertaken in which the subject performed hard work as mason and labourer for a term of ninety-five days. On a diet of about 5,000 calories, with an average of 7·22 g. of nitrogen per day, a slight loss of nitrogen resulted, namely, 34 g. for the whole period. To get the nitrogen minimum for hard work, the last ten days of the period may be taken, in which nitrogen equilibrium was maintained on 5·72 g. of nitrogen (= 35·75 g. of protein).

An important question is, naturally, whether this subject was in any way the worse for this prolonged period of minimal nitrogen diet. It must be admitted that he had lost a certain amount of nitrogenous substance, although there was every evidence that his condition was just as good as at the beginning. No period of recovery was necessary and, indeed, he was anxious to begin a new experiment.

Experiments were also made by Hindhede on himself and on a student with similar results. The former gave a protein minimum of 16 g., with a calorie value of 2,650, doing light work. The latter was doing moderate work on a diet of 3,700 calories and protein content of 25 g.

It appears that we must admit that, for a strong healthy man, the protein food actually necessary to replace wear and tear is very much less than that usually assumed. It is interesting to notice that, as would be expected, the wear and tear in hard work is greater than in moderate work, if we may judge by the rise in the protein minimum from 25 g. in the latter case to 35 g. in the former. But it is found to be the same fraction of the total intake in energy.

Effect of Carbohydrate.—In the experiments on feeding with the digestion products of proteins already referred to, it may have been noticed that, while Loewi (1902) was successful, certain other workers were unable to confirm his results. Cathcart calls attention to the fact that, in Loewi's experiments, carbohydrate was present to make up the proper calorie value, whereas in the experiments that failed, fat only was used. Further, Cathcart himself (1909) found that, if no carbohydrate was present in a nitrogen-free diet, creatine appeared in the urine, whereas it was absent when carbohydrate was given. The interpretation to be put on these experiments is that, in the presence of carbohydrate, resynthesis of creatine into some cell protein takes place, so that it would appear that some of the nitrogen lost in wear and tear can be made use of again by the aid of carbohydrate. It seems, however, from the results of Graham and Poulton (1913), that a repetition of these experiments is desirable, although Cathcart himself, with Orr (1914), points out that they do not affect his conclusions.

Other experiments confirm the necessity of carbohydrates for the synthesis of protein. It was shown by Hansteen (1899) that it applied to the higher plants and by Felix Ehrlich (1911) that amino-acids were incapable of acting as sources of nitrogen to yeast in the absence of carbohydrate.

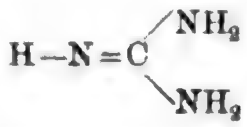
Maintenance.—Certain evidence has already been referred to which suggests that, in the wear and tear of active cells, it is not the whole of the large molecules of the nitrogenous constituents of the protoplasmic system that are broken up. One may state the fact either in the form that certain "side-chains" only of a giant-molecule or "biogen" are disintegrated, or that certain chemical individuals, forming part of the total reaction systems of the cell mechanism, are decomposed, perhaps by subsidiary reaction. Reasons have been given above (page 19) for regarding as doubtful the "biogen" view, and further evidence against it will be found on page 498, but, in the present state of knowledge, decision is impossible.

As to the fact that protoplasm itself does not break up, some additional evidence may be mentioned here. M'Collum (1911, 2) feeds pigs for a sufficient time on protein-free diet to obtain a constant ratio between the creatinine and the total nitrogen output; the total nitrogen is then taken as being that due to endogenous metabolism. The food protein to be tested is then introduced into the food in quantity equivalent to the nitrogen excreted, an isodynamic portion of the carbohydrate food being withheld. The experiments of most interest in the present connection are those with zein and with gelatine. Zein contains neither glycine,

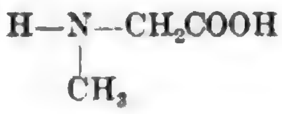
lysine, nor tryptophane, but an excess of glutamic acid; gelatine contains neither tyrosine nor tryptophane, but an excess of glycine. The animal, however, utilises the nitrogen of zein to the extent of 80 per cent., and that of gelatine to 50 or 60 per cent. This is shown by the fact that, instead of the extra nitrogen given appearing in the urine, as would happen if it were not utilised for repair, only 20 per cent. or 40 per cent. respectively is excreted. On the other hand, when zein is given, even in considerable excess over maintenance need, no evidence is obtained of the formation of new body tissue; whereas, if casein is given, 20 to 25 per cent. increase in body protein results. It seems evident that the repair processes are of a different character from those of growth. The processes of cell wear and tear and their repair do not appear to involve the destruction and resynthesis of an entire protein molecule.

In the investigation of the endogenous nitrogen metabolism, the importance of *creatine* has been pointed out, so that a few words as to its chemical nature are advisable. It may be looked upon as a substituted guanidine, in that one of the NH_2 groups is replaced by methyl-glycine. Thus:—

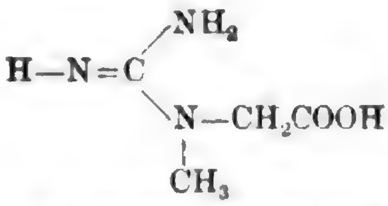
Guanidine is—



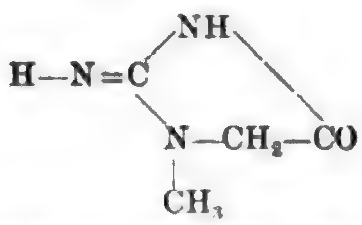
Methyl-glycine or sarcosine is—



Creatine is—



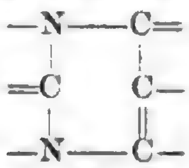
When boiled with dilute acids, it loses a molecule of water and is converted into *creatinine*, an internal anhydride, with basic properties, since the COOH group has disappeared:—



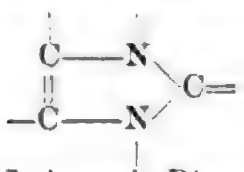
Creatinine is converted again into creatine in alkaline solution (see Bunge-Plimmer, 1907, pp. 153-155).

The method used for estimation is that of Folin (1904), which depends on the colour reaction of creatinine with alkaline sodium picrate, as described by Jaffé.

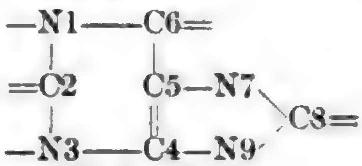
As *nucleins* are important constituents of the cell nucleus, it is to be expected that their metabolism would be chiefly of the endogenous kind. Before discussing the question, the chemical nature of these substances must be indicated. As already described, their characteristic group is the purine nucleus, the chemistry of which has been completely worked out by Emil Fischer (1882-1906). It may be regarded as a fusion of the pyrimidine and imin-azole rings, thus:—



Pyrimidine Ring.



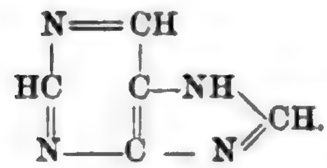
Imin-azole Ring.



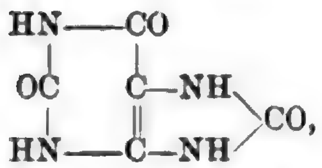
Purine Nucleus.

It will be noticed that in the purine nucleus the two component rings have two carbon atoms in common.

For convenience, each constituent of the purine nucleus is numbered, as in the formula. Purine itself is represented as:—



Uric acid is



and is described as 2-6-8 trioxy-purine. A large number of important derivatives are known, in which amino-, oxy-, or methyl groups occur in various positions.

The substances called *nucleins* are compounds of a protein with nucleic acid. This latter is itself a compound of phosphoric acid with a pentose (five carbon sugar) and a purine or pyrimidine derivative. There is a whole series of enzymes concerned in the metabolism of nucleins, according to the nature of the particular purine derivative present (see the monograph by Walter Jones, 1914).

Purine Metabolism.—Like that of proteins, is exogenous and endogenous. If we look at Folin's table reproduced on p. 94 of Cathcart's monograph (1912), we may note that, although the excretion of uric acid, taken as representing the purine metabolism, increases somewhat on a diet rich in nitrogen, the relative increase is much less than that of urea. Thus, while urea rises from 2·2 g. to 14·7 g., uric acid only rises from 0·09 to 0·18 g. This indicates, as Folin points out, that the chief source of uric acid is endogenous. In these experiments, purines were, of course, excluded from the diet, as far as possible. At the same time, if purine derivatives are given in the food, in excess of the amount required for maintenance, they are excreted. The data of Hamill and Schryver (1906) show that, on ordinary diet, there is a constant ratio between the uric acid and total nitrogen output. The organism can also form purines from ordinary proteins, as shown by their increase in the developing chick; before incubation, there are practically no purines in the egg. Although we have no evidence of such synthesis in the adult mammal, it cannot be excluded as a possibility. Moreover, the question is complicated by the fact that there are oxidising enzymes in various tissues, whose action results finally in the conversion of uric acid into urea and oxalic acid. One of the intermediate substances formed is alloxan, whose possible intervention in the process of de-amination we have seen above (page 266). Ackroyd and Hopkins (1916) have obtained evidence that arginine and histidine together may serve as sources of the purine ring.

As regards endogenous uric acid, there are two states in which increased excretion occurs, fever and severe muscular work. Both are associated with breakdown of muscular tissue, so that the uric acid seems to be chiefly derived from this tissue.

For further information, the reader is referred to Starling's book (1915, pp. 774-782).

The increased production of uric acid in severe muscular exertion leads us next to consider the question of protein metabolism in work.

NITROGEN METABOLISM IN MUSCULAR WORK

Since the endogenous output of nitrogen is to be regarded as the expression of wear and tear of the tissues, it would naturally be expected that muscular work would lead to a marked increase.

But it is a remarkable fact that, so long as the work is not excessive and does not lead to pathological conditions, there is practically no change in the nitrogen output, assuming also that the supply of carbohydrate and of oxygen are in sufficient amount.

For the various evidence bearing on this point, the reader is referred to Cathcart's monograph (1912, pp. 109-121). The work of Higgins and Benedict (1911) on the urine of the runners in one of the Marathon races may be added. They were unable to determine the absolute amounts of the various constituents,

but point out the importance of the ratio of carbon to nitrogen and of calories to nitrogen, as indicating normal or perverted protein metabolism. In twelve out of eighteen, the values were normal, in six they were high. In these six, there was practically no lactic acid and no reducing power to indicate disturbance of carbohydrate oxidation, so that the result must be considered to be due to abnormal protein metabolism. Such substances as creatinine, uric acid, amino-acids would account for the increase of the carbon to nitrogen ratio above the normal, where it is given chiefly by urea and ammonia.

There is general agreement that the source of the energy in muscular work is the oxidation of carbohydrate, which will be discussed in the next section of this chapter. At the same time, it is extraordinary that there should be so little evidence of increased wear and tear of the nitrogen-containing machinery of the cell.

What explanation can be suggested for this fact? To begin with, although excessive work may be looked upon as pathological, the fact that uric acid is increased in such a condition suggests that there is always an increase of the endogenous protein breakdown due to wear and tear, since the result of excessive work is probably to be regarded merely as an exaggeration of a particular phase of the chemical reactions involved in the whole process of contraction and restitution. Moreover, analysis of muscle itself after work has shown that the purine content is increased (Burian, 1905, M'Leod, 1899), while Brown and Cathcart (1909) and Pekelharing and Van Hoogenhuyze (1910) found an increase of creatine.

Hermann (1867, p. 100 of the first part) distinguished between two processes in muscle, the one the contraction process, by which energy is given out, associated with the production of carbon dioxide, lactic acid and a nitrogenous compound, called provisionally "myosin"; the other process is associated with a using up of the tissue itself, giving rise to carbon dioxide and creatine. The restitution of the energy-affording material of high chemical potential is effected by the aid of oxygen, and makes use of the nitrogenous product of the breaking-down process "myosin" and probably also of the lactic acid. The restitution of the tissue structure itself requires the supply of some nitrogenous material from without—Hermann says "protein," we should now prefer to say amino-acids or purines. Oxygen is of course required. The resemblance of this view to that associated with the names of Fletcher and Hopkins, which they have established by a large number of experiments, and shown that the contraction itself is a double process, is great, as we shall see later; the point to be noticed here is the difference between the nitrogenous metabolism in the two kinds of change; the normal contraction results in the separation of a substance which is used up again with the aid of energy derived from an oxidation process of some kind, whereas the wear and tear of the machine itself gives off such nitrogenous compounds as creatine or uric acid, etc., which are excreted and must be replaced by new material.

The name "*inogen*" for the complex substance of high energy content was first used by Hermann, and will be found on p. 79 of the third part of the book above referred to. The statement is made there that it was suggested in the second edition of the same author's "Grundriss der Physiologie des Menschen," Berlin, 1867.

In starvation, the heart, while continually at work, does not lose weight; so that it must be able to utilise nitrogen derived from the other tissues. In Cathcart's experiments (1909), already mentioned, the appearance of creatine in starvation was made use of to investigate the problem of resynthesis. It was assumed that its escape was due to the absence from the tissues of some material which normally caused its retention. It was found to disappear if carbohydrate food was given, but not if either protein or fat without carbohydrate was given. The appearance of creatine is, according to this worker, to be regarded merely as an index of failure of resynthesis, which process only takes place in the presence of carbohydrate.

We saw above (page 269) how important the function of carbohydrate is in the synthesis of protein, so that the hypothesis in its application to muscle is in accordance with other known facts.

As to the nature of the chemical changes concerned in this synthesis of protein under the influence of carbohydrate we have, at present, little more than suggestions. We know that simple aldehydes form compounds with ammonia, and it seems more than likely that amino-

acids combine in a similar way with reactive aldehydes or ketones, in the organism. The formation of pyruvic and glyceric aldehydes in carbohydrate metabolism, as we shall see later, is practically certain.

Knoop (1910) showed that an α-ketonic acid injected into an animal was converted into the corresponding amino-acid. This is the reverse process to one of the modes of de-amination of amino-acids, as we saw above, and is, apparently, together with the similar conversion of α-hydroxy-acids, the first step in resynthesis of protein.

CARBOHYDRATE METABOLISM

Many facts relating to this part of our subject have been referred to previously, in an incidental manner. It will have become clear that the great function of carbohydrate food is to *afford energy*. This applies not only to that given off by muscle in contracting, about which more details will be given in Chapter XIV., but also to that required to bring about endothermic reactions, an example of which we have met with in the case of nitrifying organisms.

The consumption of sugar in the active heart has been shown by Locke and Rosenheim (1907), Rohde (1910), and confirmed by others; in the intestine by Rona and Neukirch (1912). In these cases, the particular kind of sugar supplied is not a matter of indifference. Dextrose, mannose, and galactose are utilised by the intestinal muscle, and increase its activity. Fructose is said not to be consumed by this muscle, and to have no effect in increasing its activity.

In what follows I must assume that the reader is familiar with the elementary facts relating to the properties and stereochemistry of the ordinary carbohydrates; they will be found in the book of Bunge and Plimmer (1907, pp. 106-130) and from some aspects in that of L. J. Henderson (1913, pp. 222-232). The work of Emil Fischer (1884-1908) has been the chief means of our information of the constitution of the sugars; as we have seen, that of the purines and proteins is also due to him.

Since the organism must have a supply of material for energy purposes, if the more appropriate carbohydrates and fats are absent, protein is used up. This function of carbohydrates as *sparers of protein* is shown even in starvation, where the nitrogen output falls to one-third of its previous amount if cream and starch are given (Cathcart, 1909). But carbohydrate is more effective than fat; the nitrogen output, diminished by carbohydrate, goes up again on fat only. The fact is probably one aspect of the essential function of carbohydrate for protein synthesis.

PRODUCTS OF CARBOHYDRATE METABOLISM

Although the ultimate products of carbohydrate metabolism in the organism are, of course, carbon dioxide and water, it is a matter of interest, as well as of importance, to know the stages passed through, since these result in the production of reactive substances, which play an essential part in various physiological phenomena, including the processes of synthesis.

The knowledge we possess is due mainly to the work of Embden with his co-workers and of Dakin with his co-workers. To simplify description, a diagram, taken in the main from the results of these investigators, will be of service:—

d-Glucose

$\uparrow\downarrow$

Active glyceric aldehyde $\rightleftarrows$ Glycerol

$\uparrow\downarrow$

Pyruvic aldehyde $\rightleftarrows$ *d*-Alanine

$\uparrow\downarrow$

d-Lactic acid

$\uparrow\downarrow$

Pyruvic acid

$\downarrow$

Aceto-acetic acid $\leftarrow$ Acetaldehyde $\rightleftarrows$ Alcohol

$\downarrow$

(Acetic acid)

$\downarrow$

Carbon dioxide and water

Putting chemical formulæ in place of names, we have :—

$$
\begin{array}{c}
CH_2OH.CHOH.CHOH.CHOH.CHOH.CHO \\
\downarrow\uparrow \\
CH_2OH.CHOH.CHO \mid CH_2OH.CHOH.CHO \rightleftarrows CH_2OH.CHOH.CH_2OH \\
\searrow\uparrow \qquad \swarrow\nearrow \\
CH_3.CO.CHO \rightleftarrows CH_3CH(NH_2).COOH \\
\uparrow\downarrow \\
CH_3.CHOH.COOH \\
\uparrow\downarrow \\
CH_3.CO.COOH \\
\downarrow \\
CH_3.CO.CH_2.COOH \leftarrow CH_3.CHO \rightleftarrows CH_3.CH_2.OH \\
\downarrow \\
(CH_3.COOH) \\
\downarrow \\
2CO_2 + 2H_2O
\end{array}
$$

It will be noticed that most of these reactions are marked as being reversible: we shall find evidence of this by direct experiment in most cases and, as is pointed out by Embden and Kraus (1912), the processes of hydrolysis, oxidation, and synthesis are all intimately connected in carbohydrate metabolism. Carbohydrate food, when stored, takes the form of glycogen and this, hydrolysed, becomes glucose as required by the organism. Here we have clearly a reversible reaction and the fact that glucose is produced warrants our taking glucose as the starting point of our investigation.

We may now inquire what experimental evidence there is for the series of changes represented as occurring in the organism. It will be noted that some of the reactions in the numbered paragraphs include more than one step. They are numbered for convenience of future reference and the letter **R** directs attention to the fact that the reaction so marked is the synthetic aspect of the reaction with the same number.

1. *Glucose to Lactic Acid.*—Embden and Kraus (1912) showed that the liver, when poor in glycogen, produces lactic acid when blood containing glucose is perfused through it. If the liver contains much glycogen, lactic acid is given off without the necessity of adding glucose.

1. *R. Lactic Acid to Glucose.*—The previous reaction reversed. In the above experiments, if the liver was poor in glycogen and blood containing lactic acid was perfused, lactic acid was found to disappear. Further, lactic acid is converted to glucose in the dog, made diabetic by removal of the pancreas (Embden and Oppenheimer, 1912, p. 196), (Mandel and Lusk, 1906).

The various changes with which we are dealing are, in all probability, some of them certainly, carried out by the agency of enzymes. The conditions in which enzymes favour the synthetic side of reversible reactions will be discussed in the next chapter.

2. *Glyceric Aldehyde to Lactic Acid.*—Why is glyceric aldehyde

$$(CH_2OH.CHOH.CHO),$$

instead of dihydroxy-acetone ($CH_2OH.CO.CH_2OH$), indicated as the intermediate stage between glucose and lactic acid? From the action of alkali on glucose (see Dakin's monograph, 1912, p. 86) it is most probable that one or the other of these is the correct one. Embden, Baldes and Schmitz (1912) showed that washed blood corpuscles readily form lactic acid from the former, as they do from glucose, but that from dihydroxy-acetone very little is formed, *less* in fact than from glucose, so that it does not appear to be the normal process. It is remarkable that the unnatural *l*-lactic acid is formed in larger proportion than the *d*-lactic acid. The liver, when poor in glycogen, has the same effect.

It is probable that the *l*-lactic acid appeared in these experiments because the racemic glyceric aldehyde was used and the *d*-component is used by the liver to form glucose more rapidly than is the *l*-component, with which the lactic acid reaction has to be content, so to speak. On the other hand, there is evidence that di-hydroxy-acetone is more readily

fermented by yeast than is glyceric aldehyde, so that, in this case, it may be the intermediate stage; although lactic acid itself does not seem to be so (see Harden's monograph, 1911, pp. 90-94).

2. *R. Lactic Acid to Glyceric Aldehyde.*—I am not aware that this change has, directly, been shown to occur. But, of course, if glyceric aldehyde is an intermediate stage, it must do so, since lactic acid is converted to glucose, as we have seen.

3. *Glucose to Glyceric Aldehyde.*—This reaction also has not actually been shown to happen, but the same argument as above applies.

3. *R. Glyceric Aldehyde to Glucose.*—Embden, Baldes and Schmitz (1912, p. 127) have brought evidence to show that the liver performs this reaction.

4. *R. Glycerol to Glucose.*—Confirmatory evidence of the importance of glyceric aldehyde is afforded by the behaviour of glycerol. Lüthje (1904) showed that the diabetic animal can form glucose from glycerol, and Schmitz (1912) found that glycerol, added to blood perfused through the liver, diminished; although if the liver were rich in glycogen, this did not occur.

5. *Lactic Acid from Glycerol.*—Oppenheimer (1912) showed that lactic acid is formed from glycerol by perfusion through the glycogen-free liver. The obvious way from glycerol to lactic acid is by glyceric aldehyde, as a stage of oxidation, so that the way to glucose is also, no doubt, through the same substance.

5. *R. Glycerol from Lactic Acid.*—Embden, Schmitz, and Baldes (1912, p. 185) showed that the liver, perfused with glyceric aldehyde, forms glycerol, so that this reaction, again, is a reversible one.

6. *R. Alanine from Pyruvic Acid.*—As already mentioned, the formation of alanine from pyruvic or lactic acid has been shown by Knoop (1910), and by Embden and Schmitz (1910).

6. *Lactic Acid from Alanine.*—Neuberg and Langstein (1903) obtained this result, so that the above reaction is reversible. Neuberg and Langstein's reaction is interesting as one of the first definite cases of de-amination in the animal organism. The reaction, no doubt, passes through the stage of pyruvic aldehyde.

7. *Pyruvic Acid to Lactic Acid.*—Paul Mayer (1912) found that sodium pyruvate in excess, administered subcutaneously, gave rise to both glucose and lactic acid in the urine. Embden and Oppenheimer (1913) found that large amounts of lactic acid were produced by perfusion of the glycogen-free liver with pyruvic acid.

8. *Pyruvic Acid to Glucose.*—See number 7 above. A. I. Ringer (1913) also found that pyruvic acid yields glucose in the organism; but in certain cases it was found that the amount obtained was much less than when corresponding amounts of lactic acid or alanine were given. Pyruvic acid, apparently, is not a necessary intermediate stage in the conversion of alanine into glucose. What the intermediate stage is will appear presently. Dakin and Janney (1913) state that pyruvic acid is only converted to glucose when the conditions are such as to favour its initial reduction to lactic acid, which is the necessary intermediate stage.

9. *Pyruvic Aldehyde to Lactic Acid.*—Pyruvic aldehyde is sometimes, not quite correctly, called methyl-glyoxal, but it cannot chemically be regarded as derived from glyoxal $\left(\begin{array}{c}CHO\\ |\\ CHO\end{array}\right)$ by replacement of a hydrogen atom in an aldehyde group by methyl. Although Embden and Oppenheimer (1913) do not think that this substance is an intermediate stage between glucose and lactic acid on account of its not being optically active, recent work by Dakin and Dudley (1913, 1, 2, 3) indicates that it has, to say the least, considerable importance. These observers find that there is present in nearly all tissues, especially in the liver and muscles, an enzyme, glyoxalase, which acts with great rapidity on "glyoxals" of various composition, transforming them into lactic acid compounds. The presence of this enzyme strongly suggests that pyruvic aldehyde is an intermediate stage between glucose and lactic acid and it might well come in between glyceric aldehyde and lactic acid in the scheme given above. The fact that it does not possess an asymmetric carbon atom and that, on this account, there are not two optical isomers, as in lactic acid and in glyceric aldehyde, is no serious objection to the

view of its importance as an intermediate substance between them. Dakin, indeed, thinks that the fact of its optical inactivity is favourable to the synthesis of dextrose through glyceric aldehyde. Suppose that both optical isomers of lactic acid be present, then, if converted first into pyruvic aldehyde, dextroglyceric aldehyde may be formed from both, by an appropriate optically active catalyst, and, from this, dextrose. In fact, *l*-lactic acid, the unnatural form, readily yields glucose in the animal organism, when rendered diabetic by phloridzin (Dakin and Dudley, 1913, 2, p. 129). We shall see later that an optically active catalyst is able to form, from optically inactive substances, a preponderance of one optical isomer of an optically active product. When acting on pyruvic aldehyde, glyoxalase yields a mixture of the two forms of lactic acid, but in unequal proportion, and the authors think that two enzymes are concerned, since an enzyme preparation, giving, when fresh, a preponderance of the lævo-acid, after standing, gave an excess of the dextro-acid, when acting on a new supply of the substrate. Glyoxalase appears to have a wide distribution; it has been found in the oyster and in yeast. It is absent from the pancreas and a substance is present in extracts of this gland which has the power of actually inhibiting the action of glyoxalase (Dakin and Dudley, 1913, 3). These facts are significant in view of the profound relation between the pancreas and carbohydrate metabolism.

9. *R. Lactic Acid to Pyruvic Aldehyde.*—Dakin and Dudley (1913, 1) showed that lactic acid is readily converted into pyruvic aldehyde by digestion with nitro-phenyl-hydrazine. Further, that in faintly acid solution both lactic acid and alanine are decomposed with formation of pyruvic aldehyde:

$$CH_3.CHOH.COOH \rightarrow CH_3.CO.CHO + H_2O$$
$$CH_3.CH.NH_2.COOH \rightarrow CH_3.CO.CHO + NH_3$$

By the action of glyoxalase, then, lactic acid can be obtained from alanine through the intermediation of pyruvic aldehyde. With the exception of the direct conversion of pyruvic aldehyde to alanine, all the reactions involving the interconversion of glucose, pyruvic aldehyde, lactic acid, and alanine are shown to be reversible and the authors named have obtained the analogous synthesis of glycine from glyoxal.

Acetaldehyde. — Neubauer (1909) showed that α-ketonic acids are changed in the organism into the ordinary fatty acid with one less carbon atom; so that pyruvic acid will go into acetic acid. In this process it does not seem possible that any intermediate stage other than that of acetaldehyde would be passed through.

According to Neuberg and Karczag (1911) yeast juice ferments pyruvic acid with the production of carbon dioxide and acetaldehyde. Masuda (1912) found that the liver, perfused with blood containing alcohol, forms aldehyde and Embden and Baldes (1912) that the reverse change from acetaldehyde to alcohol also takes place, even in the presence of oxygen.

There is no evidence that *ethyl alcohol* is a direct stage in the oxidation of glucose in the animal organism, but it appears that acetaldehyde may well be a stage in the formation of alcohol from sugar in fermentation, although it does not seem to be a necessary one. Alanine is fermented by yeast with formation of alcohol, carbon dioxide, and ammonia and the most likely stages seem to be pyruvic acid and acetaldehyde.

Mention may be made of the fact that, under certain conditions, probably of deficient oxidation, ethyl alcohol may be obtained by distillation of various tissues, especially muscle. The possibility of absorption from the alimentary canal seems to have been excluded in some of these experiments, although it must be admitted that it does not appear to be an easy matter to be certain that it is so.

Aceto-acetic-acid is produced by the liver from pyruvic acid (Embden and Oppenheimer, 1912). It must be formed by aldol condensation from acetaldehyde, through β-oxy-butyric acid.

"*Aldol condensation*," the reader may be reminded, is simply the union of two molecules of an aldehyde, which may be effected, for example, by the action of strong hydrochloric acid, thus:—

$$CH_3CHO + CH_3CHO = CH_3CH(OH)CH_2CHO.$$

Aceto-acetic acid is found in the urine in certain pathological states associated with disturbed carbohydrate metabolism and has been found by Masuda (1912)

to be formed by the liver from ethyl alcohol through the intermediate stage of acetaldehyde; so that acetaldehyde is, as it were, the meeting place of two reactions, both leading to aceto-acetic acid, the one from pyruvic acid, the other from ethyl alcohol.

The further oxidation of aldehyde to *carbon dioxide and water* is probably through *acetic acid*, as suggested by Neubauer's change of pyruvic into acetic acid in the organism. This would then be the chief reaction; those leading to alcohol or to aceto-acetic acid diverging at the acetaldehyde stage.

Another mode of oxidation of glucose should be referred to, namely, that to *glucuronic acid*, in which the CH_2OH group of glucose is converted into COOH. Further stages of oxidation would yield saccharic and oxalic acids. Camphor, administered to an animal, is excreted in combination with glucuronic acid (Musculus and von Mering, 1875). That this acid arises from oxidation of glucose is shown by the experiments of Paul Mayer (1902), who found that, in inanition, in which very little glycogen remains stored up, scarcely any glucuronic acid was excreted on administration of camphor; whereas, if glucose was administered at the same time, the usual amount was obtained. It is doubtful whether, normally, further oxidation takes place along this path, since oxalic acid is only oxidised with great difficulty in the organism (Dakin, 1912, p. 45). At any rate, this mode of oxidation of glucose is not the chief or normal one.

Diabetes.—If the pancreas be removed, and in some pathological conditions, large quantities of glucose are excreted by the kidneys. A remarkable fact is that, even after all carbohydrate stores are used up and none is given in the food, the organism breaks down body-protein in order to form glucose. From the experiments of Cathcart (page 269 above) we have seen the necessity of carbohydrate for protein synthesis and the facts of diabetes suggest that the cells imperatively demand carbohydrate. It is interesting to note that, even after a prolonged fast, sugar is never absent from the blood of the normal animal.

The experiments of Lusk (1910) have shown that glycine, alanine and three of the carbon atoms in aspartic and glutamic acids are converted into glucose in the organism and that 100 parts of meat can give 58 parts of glucose. Since the reaction is, doubtless, reversible, the possibility of production of various amino-acids from glucose is shown. For further information on the question of diabetes see Starling's "Human Physiology" (1915, pp. 800-809).

THE FUNCTION OF CANE-SUGAR IN THE PLANT

According to Parkin (1911), saccharose is the sugar of most importance in the plant, both as reserve carbohydrate and as circulating sugar. It serves, in fact, as regards carbohydrate, much the same purpose as asparagine in respect of protein metabolism (Horace T. Brown, 1906). Saccharose has properties that fit it especially for such purposes. It is very soluble and yet easily crystallises. It is easily hydrolysed by acids and by invertase. It has no reducing properties, since the aldehyde group is not functional. It appears that it can be condensed to starch, without previous hydrolysis, and probably also to cellulose.

THE METABOLISM OF FAT

The fats taken as food, or found in various situations in the body of the organism, are the tri-glycerides of the higher fatty acids, sometimes accompanied, as in milk, by small amounts of the glycerides of the lower fatty acids, butyric, caproic, etc. The acid may be either a saturated one, as stearic, or an unsaturated one, such as oleic, in which there are carbon atoms united by double bonds ("ethylene linkage").

The substances known as "lipoids," which were described above (page 130), are also found in the tissues. The function of these in the formation of the cell membrane has also been discussed.

Formation of Fat in the Organism

1. *From Fat in the Food.*—If not oxidised for energy needs, fats taken as food appear to be deposited in the tissues without change. Lebedev (1882) fed dogs, which had lost the greater part of their fat from inanition, either on a diet containing mutton fat in considerable amount, or on a similar diet containing linseed oil in place of the mutton fat. After some weeks, it was found that the

fat of the dog which had received mutton suet was solid at 50° C., whereas that of the dog fed on oil was still liquid at 0° C. Although fats are hydrolysed in the lumen of the intestine, their constituents are resynthesised in the wall of the intestine, and are carried in the chyle to the blood as neutral fats. The part played by enzymes in this process will be discussed later.

2. *From Carbohydrate in the Food.*—Although the formation of fat from carbohydrate in the process of fattening animals for food seems obvious in the ordinary practice of farmers, complete evidence was wanting until the experiments of Lawes and Gilbert (1852, p. 350) on young pigs, fed on barley, in which it was shown that the amount of fat laid on was considerably greater than could possibly have come from the protein in the food, after deducting that used for tissue formation in the body. The amount of fat in barley food is very small.

Until the recent work of Miss Smedley (1912) the chemical mechanism of such a transformation was unknown.

As regards the glycerol component, the facts described in the previous section show how it is readily obtained from glucose.

As regards the fatty acid component, the first fact to be noticed is that the fats of the organism are limited to those whose fatty acids contain an even number of carbon atoms. There must obviously be some reason for this. Another fact is that the process of formation from carbohydrate is, as Leathes has shown (1906, p. 85), an exothermic one. This might, at first sight, seem surprising, since the heat of combustion of a gram-molecule of fat is so much higher than that of a gram-molecule of glucose. But we must remember that several molecules of sugar are required to form one of a higher fatty acid; for example, stearic acid contains eighteen carbon atoms.

Miss Smedley and Miss Lubrynzka (1913, 1 and 2) have brought forward good evidence to show that the process of fat synthesis in the organism takes place in the following way: We have seen that pyruvic acid is formed as a stage in the oxidation of glucose. Moreover, there is reason to believe that pyruvic acid is converted by a further process, probably enzymic, into acetaldehyde and carbon dioxide. This aldehyde may then condense with another molecule of pyruvic acid to form a higher ketonic acid, thus:—

$$CH_3CHO + CH_3CO.COOH = CH_3CH{:}CH.CO.COOH + H_2O.$$

The investigators named have succeeded in obtaining this reaction with butyl aldehyde and pyruvic acid.

The next stage is the conversion of the ketonic acid thus obtained into its aldehyde and carbon dioxide, by a similar process to that by which the acetaldehyde was originally obtained from pyruvic acid:—

$$CH_3.CH{:}CH.CO.COOH = CH_3.CH{:}CH.CHO + CO_2.$$

This aldehyde, which has two more carbon atoms than that from which we started, condenses with another molecule of pyruvic acid, forming a ketonic acid of still longer carbon chain, and so on.

From the unsaturated ketonic acid formed at any stage we obtain, by oxidation, an unsaturated fatty acid with one less carbon atom, thus:—

$$CH_3.CH{:}CH.CO.COOH + O = CH_3.CH{:}CH.COOH + CO_2,$$

and from this, by reduction, the fatty acid containing two more carbon atoms than the aldehyde from which we started in this particular stage. For example:—

$$CH_3.CH{:}CH.COOH + H_2 = CH_3.CH_2.CH_2.COOH,$$

that is, we have obtained butyric acid starting from acetaldehyde.

The process may be repeated many times, and fatty acids with a long chain of carbon atoms obtained. It is necessary to remember, however, that branched chains are not formed in this comparatively simple manner.

The reverse change from *fat to carbohydrate* is known to occur in the germination of fatty seeds, where starch and cellulose are formed from the fat.

In hibernating mammals, as Pembrey (1903) has shown, there is an extraordinarily low respiratory quotient of 0·3-0·4. This means that there is a conversion of substances containing a small amount of oxygen into others containing a larger amount of oxygen, that

is, fat into carbohydrate. It can be shown by the increase of weight that oxygen is actually retained. When a marmot converts the carbohydrate of its food into fat, preparatory to hibernation, the respiratory quotient is high.

This numerical quantity, the *respiratory quotient*, has not yet been explained in these pages. It is simply the ratio of the volume of the carbon dioxide given out to that of the oxygen taken in. Carbohydrate may be looked upon as consisting of carbon plus water, so that, if this were the only material oxidised in the organism, the oxygen would have been entirely used to combine with the carbon and the respiratory quotient would be unity. In fat, on the other hand, besides the carbon there is also hydrogen to be oxidised, as easily seen by the formula, say of palmitin, $C_{51}H_{98}O_6$. Part of the oxygen taken in is used for the oxidation of hydrogen, so that there is *less* carbon dioxide given out than that equivalent to the oxygen taken in and the respiratory quotient is less than unity. The determination of the respiratory quotient enables us to see what is being oxidised, when different diets are given.

3. *Fat from Protein in the Food.*—Although amino-acids are de-aminated in the organism, so that pyruvic acid is formed from alanine, and, from pyruvic acid, as shown in the preceding section, higher fatty acids can be synthesised, it is a remarkable fact that all evidence tends to show that no fat is laid on by the organism, however large a diet of pure protein is taken. The effect is simply to increase the nitrogenous and general metabolism.

In certain toxic conditions there appears at first sight to be a change of the protoplasm of the cells, especially of the liver, into fat. But careful investigation has shown that there is no actual increase of the total fat of the body; what happens is that fat from other parts migrates to the liver and there is also some kind of aggregation of the lipoids of the protoplasm, so that, from being invisible as a distinct phase, they become particles or droplets, readily seen under the microscope. Fat is formed from protein in developing eggs (M'Clendon, 1915).

That fats can be used as *sources of energy*, in muscular contraction, for example, is shown by the respiratory quotient. We have just seen that, if fat is being consumed, this value falls. When muscular work is performed with a diet consisting almost entirely of carbohydrate, the respiratory quotient is 0·9; when fat is exclusively taken, it falls to 0·72. This shows that in the latter case a substance containing oxidisable hydrogen as well as carbon, that is, fat, is being consumed.

The chemical processes taking place in the utilisation of fat are not definitely known. But it is altogether probable that the process of synthesis from lower fatty acids, described above, goes also in the reverse direction and, when arrived at, these acids are quickly oxidised. There is also direct evidence of the breakdown of fats into aceto-acetic and β-oxy-butyric acids, together with acetone, in diabetes, where they appear on a diet formed exclusively of fat and protein. In the normal organism, an exclusively fatty diet, continued for some days, has been found to give rise to large quantities of these partially oxidised products.

PYRUVIC ACID

It will have been noticed how frequently this compound makes its appearance in various metabolic processes. It is interesting to collect together these facts. It is converted into alanine by a reversible reaction. It is a stage in the oxidation of glucose, so that all the substances contained in the scheme on page 273 above can be obtained from it. Further, Miss Smedley's work has shown how higher fats can be synthesised by starting from pyruvic acid. Fats, carbohydrates, and proteins, therefore, come into connection at this meeting place.

We saw above that the living cell of Aspergillus is able to form several different amino-acids from nitrate. It was pointed out, also, that if the appropriate a-ketonic or hydroxy-acid were available, the corresponding amino-acid could be formed by the liver. But the only such acids known to be present in the organism are pyruvic and lactic, from which alanine alone is formed directly. Miss Smedley's work, however, has shown how an unsaturated a-ketonic acid with two more carbon atoms can be obtained from pyruvic. From this, by addition of hydrogen, the saturated ketonic acid may be formed, and therefore the amino-acid. But the process has its limitations, since it only gives us a straight chain of carbon atoms, while leucine, for example, has a branched chain. See also Ringer and Lusk's results, page 256 above.

ANALYSIS OF METABOLIC PROCESSES

The reader will have noticed that many of the reactions described as stages in the metabolism of proteins, fats, or carbohydrates rest upon evidence derived either from perfusion of isolated organs or from experiments with extracts of tissues *in vitro*. Some investigators appear to doubt whether legitimate conclusions, as regards the processes taking place in the organism as a whole, can be drawn from experiments of this kind.

It does not seem to me that such criticism is justified. Investigation of the excreta compared with the ingesta, valuable as the information is for certain purposes, gives us very little knowledge of the chemical reactions by which the latter are converted into the former. The comparison of the organism to a town, where various occupations are carried on, is often made. If we notice that a large quantity of milk goes into the town and that a corresponding amount of cheese comes out, we conclude that the milk has been used to make the cheese, but we learn nothing about the method employed. Still less is learned by such methods with regard to the more intellectual occupations, such as that of the poet or musician. Hopkins has made use of the simile of a conjuror, who puts a loaf into a hat and takes out a rabbit. What we want to know about are the intermediate stages between the loaf and the rabbit.

Looking at the question from another point of view and taking, for example, the oxidation of glucose, it is surely permissible to consider what are the possible chemical changes that might take place. Suppose then that we find certain of the possible reactions to be brought about by extracts of tissues, while others, also chemically possible, are not; we are, it seems to me, justified in stating that the former is the way in which the organism works, at all events until the contrary has been actually proved.

Again, as to perfusion experiments: when pyruvic acid is added to blood passing through the liver and alanine is found in the issuing blood, it cannot be denied that, even in the whole organism, if pyruvic acid be present in the portal blood, alanine will be found in the blood of the hepatic vein. If it be objected that alanine might come from the substance of the cells themselves, it may be pointed out that, when the related α-ketonic acid of butyric acid is perfused, α-amino-butyric acid is formed. It seems extremely improbable that the permeability of the cells should be affected by closely related ketonic acids in such a way that the corresponding amino-acid, and this only, is washed out of them.

Since the reactions under discussion are reversible, take for example that between lactic acid and glucose, it is scarcely credible that lactic acid should wash out or cause the cells to give up glucose, while glucose causes them to lose lactic acid.

STORAGE

The organism is capable of storing, in some form or other, the chief classes of food-stuffs, although in different degrees.

Carbohydrates.—These are stored, mainly in the form of glycogen, in the liver and muscular tissues for the most part. Glycogen, being an insoluble substance, is kept out of the risk of undesirable participation in chemical reactions; on the other hand, while soluble carbohydrate is required, the action of the enzyme, amylase, converts it into maltose or glucose. In the plant, starch is the most common form of stored carbohydrate, but saccharose is very frequently met with. Soluble carbohydrates do not appear to be stored in the animal, at all events in more than very small amounts.

Fat is stored in practically all cells in larger or smaller quantity, frequently in the form of lecithin, or related substances. The main store of neutral fat is in the subcutaneous connective tissue. In the plant, neutral fats are found in some fruits and practically only in fruits.

Proteins.—It is impossible to name any particular form in which nitrogen is stored. Protoplasm in general is capable of increase and, in starvation, as we have seen, the less important tissues give up amino-acids for the benefit of the more vital organs, thanks to the presence of autolytic enzymes. Whether there

is any special form particularly adapted for the purpose of storage is at present doubtful, although certain observers believe that they have evidence of some such substance (see Cathcart's monograph, 1912, pp. 58 and 79).

Berg (1914), and Berg and Cahn-Bronner (1914) describe the presence of granules of protein material (not protoplasm) in the liver cells of well-fed animals, particularly after feeding with amino-acids. These are regarded as storage of nitrogenous substance.

Noel Paton (1910) has brought evidence, depending on the proportion of creatine to total nitrogen excreted, to show that, in fasting, after an abundant protein diet, it is mainly non-muscle protein that is first used up. The value of the evidence depends, of course, on the constant percentage of creatine contained in muscle.

Other investigators hold that there may be a storage of nitrogen in some form which has a simpler constitution than protein. In making this distinction between protein nitrogen, and that in simpler form ("extractives"), it may be noted that we assume that any nitrogen stored otherwise than as cell protoplasm does not form an integral part of a "giant" protoplasmic molecule, or "biogen," as a purely chemical system.

It was mentioned above that van Slyke and Mayer found that amino-acids, introduced into the blood, disappeared rapidly therefrom. In a further paper (1913, 1), they show that these substances are taken up by the tissues in a form such that they can readily be washed out again, even by cold water. There is, moreover, a definite equilibrium established, so that the blood, even in starvation, still contains 3-8 mg. per 100 c.c. This points to an adsorption process, especially since the alternative hypothesis mentioned by the authors, that compounds like those supposed to be prepared by Pfeiffer and Modelski between amino-acids and neutral salts, is improbable, on account of the fact that there is no evidence for the existence of such compounds, as I have shown (Communication to Biochem. Society, not yet published).

A word may be said here with respect to the experiments of Grafe and Schläpfer (1912), already referred to, in which, on feeding animals with a diet containing ammonium salts as the only source of nitrogen, a diminution of nitrogen output occurred. The conclusion drawn seems to be that protein can be synthesised from carbohydrate and ammonia. We have seen, indeed, that, from pyruvic acid and ammonia, alanine can be formed in the organism, but evidence is wanting as to other necessary amino-acids. Admitting the possibility of the requisite α-ketonic acids being formed from carbohydrate, the retention of nitrogen from ammonium salts, if utilised for synthesis of protein, should not be observed if the diet is free from carbohydrate. Accordingly, Taylor and Ringer (1913) made the experiment. They found that, even in these conditions, nitrogen was retained from ammonia and they are of the opinion that the evidence points to the reversal of the process of de-amination as the explanation of the phenomenon. This reaction, if an oxidation, may be presented thus:

$$K = \frac{(\text{ketonic acid}) \times (NH_3)}{(\text{amino-acid}) \times (\text{oxygen})}$$

where K is the equilibrium constant and the factors in brackets are the concentrations of the four components of the system. Increase of ammonia leads to diminution of ketonic acid and this again involves increase in amino-acid, which may be utilised in the organism. Alanine, for example, obtained in this way, may be used for the same purposes as that derived from proteins, so that the latter are spared from breaking up.

Support is given to this view by the later experiments of Grafe (1913), in which it is shown that retention of nitrogen can be obtained when urea is given as food. It is only necessary to assume that the reaction by which ammonia is converted into urea is also reversible, and we see that excess of urea involves increase of ammonia, and we have the same phenomenon as when ammonia itself is given. The fact that when either ammonia or urea is injected subcutaneously, it is entirely excreted by the kidneys, without giving rise to any retention of nitrogen, suggests that the concentration in which it arrives at the de-aminating tissues is too small to result in any perceptible mass action.

OPTICAL ACTIVITY

It is well known that compounds containing one or more carbon atoms each united to four different atoms or groups rotate the plane of polarised light, on account of their asymmetry.

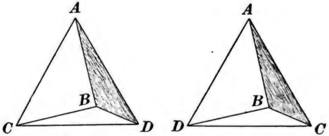


FIG. 74. DIAGRAM OF ASYMMETRIC CARBON ATOM.

A, B, C, D represent four different groups, attached to the four angles of a tetrahedron, which stands for a carbon atom. The two figures are mirror-images of one another, and cannot be made to coincide.

(van't Hoff, 1901, ii. p. 97.)

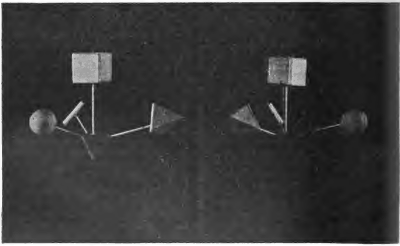
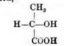


FIG. 75. PHOTOGRAPH OF A MODEL OF AN ASYMMETRIC CARBON ATOM, TOGETHER WITH ITS IMAGE IN A MIRROR.—The four solids of different shape represent the four different chemical groups. The reflected image can be detected by the double outline in places.

The theory of the asymmetric carbon atom is due to Le Bel (1874) and van't Hoff (1874). If we take lactic acid,



$$\begin{array}{c} CH_3 \\ | \\ H-C-OH \\ | \\ COOH \end{array}$$

we see that the central carbon atom is united to four different groups. The compound is accordingly optically active.

Since a large number of the compounds of physiological interest are of this nature, the method by which the changes of their concentrations are investigated by measurement of their power of rotating the plane of polarised light is a very valuable one. The instrument used is called "*polarimeter*" and details of its construction will be found in Findlay's book (1906).

Returning again to lactic acid, we find that, when prepared from muscle, it rotates the plane of polarised light to the right and is hence called dextro- or *d*-lactic acid. On the other hand, when cane-sugar is fermented by certain bacteria, a lactic acid is obtained which rotates to the left and is hence called lævo- or *l*-lactic acid. It will be obvious that a mixture of the two in certain proportions will rotate equally in both directions and appear to be optically inactive; such a mixture is called inactive or *dl*-lactic acid.

Now we must remember that the diagrams used on paper to represent chemical structure are in one plane, whereas the compounds themselves are in space of three dimensions. In order, therefore, to understand the relationship of these "optical isomers," as they are called, on account of the identity of their chemical composition, we must endeavour to represent them in space, remembering that we can only use conventional diagrams. The simplest way is to place the four groups at the points of a tetrahedron, represented in perspective, for the case of lactic acid, in Fig. 74. It will be found impossible to turn these figures in any position so as to make them coincide, in fact one is the image of the other seen in a mirror. The photograph on p. 180 of Wade's book (1905) and Fig. 75 (page 282) represent this fact.

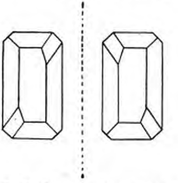
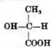


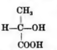
FIG. 76. CRYSTALS OF THE TWO OPTICAL ISOMERS OF AMMONIUM HYDROGEN MALATE.—Although the geometrical form is the same, they cannot be made to coincide, and are, in fact, mirror-images of one another.

(van't Hoff, 1901, ii. p. 98.)

Since, however, it is awkward to use these perspective figures, it is customary to represent them by their projections on a plane surface, thus :—



$$\begin{array}{c} CH_3 \\ | \\ OH—C—H \\ | \\ COOH \end{array}$$



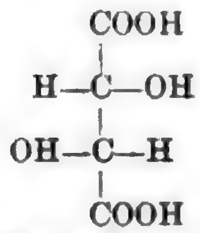
$$\begin{array}{c} CH_3 \\ | \\ H—C—OH \\ | \\ COOH \end{array}$$

bearing always in mind that such formulæ are not to be supposed to be removed out of the plane of the paper, so that, although one can be slid over the other it must not be taken up and placed face downwards. This is, of course, merely a convenient arrangement, in order to avoid the inconvenience of using solid figures.

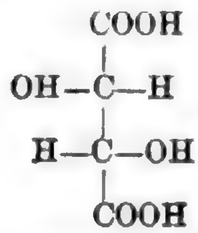
The real existence of asymmetry of such a kind that one of the isomers is the mirror-image of the other may, perhaps, be made clearer by Fig. 76, which represents crystals of the *d*- and *l*- forms of ammonium hydrogen malate. These would be said to have the same shape, but they cannot be made to coincide, and are, in fact, mirror-images of one another. They are sometimes called "enantiomorphic" forms of hemihedral crystals.

Optical isomers are also called "optical antipodes," but a mild protest must be made against the use of "antipode" in the singular, as if "antipodes" were the plural of an English word.

When there are two asymmetric carbon atoms, as in tartaric acid, we have the possibility of a further complication; thus, to begin with :—

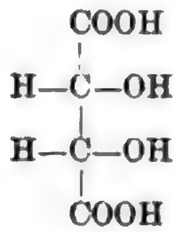


d-tartaric acid.



l-tartaric acid.

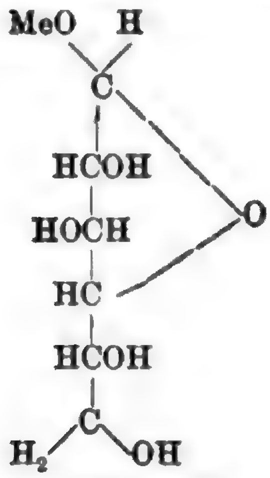
A mixture of these is found in grapes and is sometimes called racemic acid. But there is another possibility of an inactive acid, in which the asymmetry, represented in the above figures, mutually compensates itself in one single molecule :—



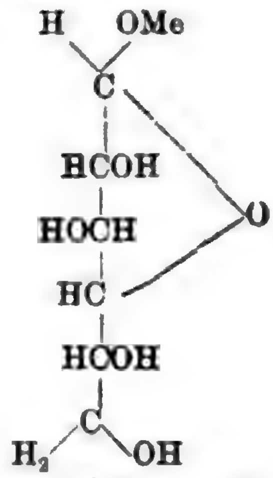
this is known as meso-tartaric, or internally compensated tartaric acid.

In the representation of the formulæ of the tartaric acids, it is to be understood that the two asymmetrical carbon atoms are supposed to be looked at from opposite ends; they will then appear alike and rotate in the same direction.

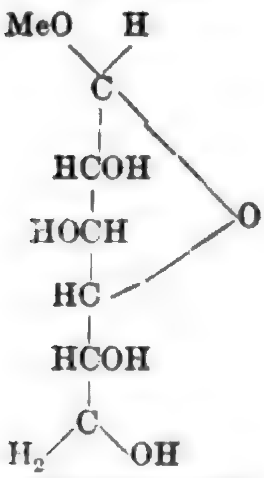
There is another class of optical isomers which must not be confused with those which are real mirror-images of one another. For example, in the α- and β-glucosides of *d*-glucose it is only the aldehyde ends that are mirror-images of one another; the other parts of the molecules are identical. The properties of isomers of this kind are therefore different, and they can be separated by solubility, etc. The rotatory power of the one is also not equal and opposite to that of the other. The real mirror-image of α-methyl-*d*-glucoside is α-methyl-*l*-glucoside. These have the same numerical values of optical rotation, but in opposite directions, they have the same melting point, the same solubility and the same external form of crystals, as shown by Fischer (1909, pp. 741-742). The following formulæ will make the relationship clear :—



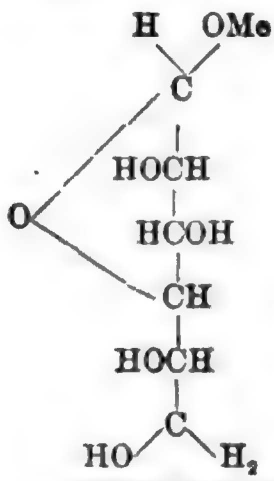
α-methyl-d-glucoside.



β-methyl-d-glucoside.



α-methyl-d-glucoside.



α-methyl-l-glucoside.

It is a remarkable fact that the structures of living organisms are composed only of the one series of optical isomers, when there is the possibility of both. The amino-acids are all of the *l*-series, the starches and sugars of the *d*-series, and so on. Not only so, but in the use of food-stuffs even for energy purposes, there is a decided preference for the same series.

Much has been made of this fact in connection with that of the inability of pure chemical methods alone to produce anything but optically inactive mixtures of the two optical isomers. It is true that all the ordinary means used, for convenience, to separate the two components involve the use of vital agency at some stage or other, but there are several considerations which seem to me to indicate that it is possible to lay too much stress on the argument. We will mention them briefly:—

1. When an optically active substance is synthesised under the agency of an optically inactive catalyst, such as hydrochloric acid, a mixture of both isomers is formed. On the other hand, if the catalyst is an optically active one, only the one isomer is formed; or, at all events, it is in great preponderance.

For example, from glucose and methyl alcohol, by the action of hydrochloric acid, the two optically isomeric α- and β-methyl-glucosides are produced. When the enzyme, emulsin, is used, the β-glucoside is formed, and by another enzyme, maltase, the α-glucoside. The reader is recommended to consult the paper by Fajans (1910) with respect to the question of asymmetrical catalysis, both by enzymes and other optically active substances. Of course, it may be said that all optically active catalysts were originally produced by vital agency, but the point here is that a chemical substance, not actually living, is able to form new optically active material, provided that it is itself optically active.

In connection with the production of other asymmetrical compounds by the aid of those already existent, the work of Erlenmeyer (1914) is of importance. By the action of *d*-tartaric acid on benzaldehyde in alcoholic solution, a lævo-benzaldehyde was obtained. Other papers by the same worker may be found in *Biochemische Zeitschrift* (Band 64).

2. Although organisms show a preference for one isomer, they are not incapable of using the other one. In the classical experiment of Pasteur (1858) of separating the two tartaric acids by the action of moulds, the *d*-acid is used up first, so that the *l*-acid can be separated from the culture after the *d*-acid is used up. But, if the experiment be allowed to continue, it was found that the rotation began again to diminish, owing to the consumption of the *l*-acid. This fact is sometimes forgotten. Similar cases have been described in the utilisation of amino-acids by fungi.

These and other cases will be found given in my monograph on "Enzyme Action" (1919, 1, p. 137). It may be mentioned here that this capacity of utilising both isomers is not confined to fungi. Parnas (1911) has shown that the rabbit can utilise *l*-lactic acid when the inactive mixture is given, although, given alone, this acid itself is toxic and is excreted.

3. In such cases, the question arises as to how far the use of what we may call the "foreign" isomer is merely for energy purposes, or whether new tissue is formed from it. If the latter, it must obviously be converted into the opposite isomer in some way. This is not an impossible occurrence. If *l*-leucine be heated with baryta water at 180°, it is converted into *dl*-leucine, or racemised. That is, half of it is changed from the *l*- to the *d*- form. As we shall see in the next chapter, there are present in organisms, in the form of enzymes, more active catalytic agents than alkalies or acids.

4. When we produce artificially, in the laboratory, an inactive mixture, it is not that the chemical reaction is unable to produce the substance that the "vital" reaction does, but that the former produces the opposite isomer in addition.

5. There is considerable evidence to show that, when an enzyme appears to deal with one optical isomer alone, it is not absolutely inactive with respect to the opposite one; there are many differences of degree in this respect (see the paper by Fajans, 1910, and the monograph by myself, 1919, 1, p. 137).

6. It is quite conceivable that optically active products might be obtained by allowing a reaction to proceed under the influence of some asymmetrical external force, say, for example, a photo-chemical reaction under polarised light; although attempts to do so have not yet met with success.

It is not easy to see what is the advantage to the organism of this undoubted preference for particular optical isomers. We have to remember that the existence of asymmetric carbon atoms is geometrically unavoidable. The enzymes which act on such compounds will probably also be themselves optically active, and the rate of action on one kind of optical isomer will no doubt be greater than that on the opposite one. A certain economy in the number of enzymes necessary is effected by limiting them to those required for one set of optical isomers, but it is scarcely to be supposed that this can be of much consequence, and it would seem indeed that more is lost than gained in the process. The replacement of an enzyme acting on one isomer by that acting on the opposite one, moreover, does not appear to be of much difficulty. Currie (1911) found that, of various pure cultures of *Bacillus bulgaricus* (the vigorous Bulgarian lactic acid organism), obtained from different sources, some formed *d*-lactic acid alone, others a mixture of *d*- and *l*- forms, and one culture produced *l*-lactic acid alone.

We must suppose that the external forces, under which the asymmetric carbon compounds forming the basis of living protoplasm were produced, were in some way or other themselves asymmetric. At that geological period, the synthesis may have received a bias in one direction, which has naturally been adhered to, as more and more elaborate compounds were evolved. On this view, the preference of one isomer over the other is, as it were, a matter of chance as to which happened to be first produced by the particular direction of the asymmetrical force.

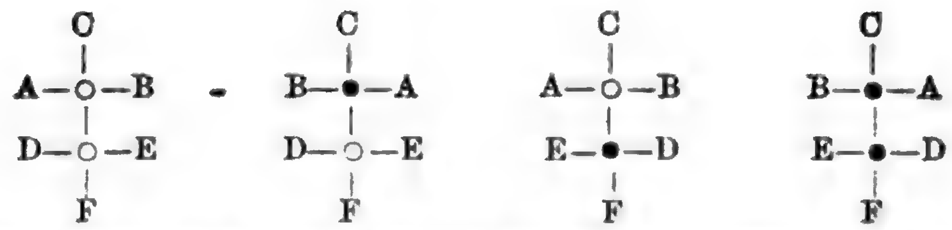
Recent work by Emil Erlenmeyer (1913), however, suggests a possible way of separating the constituents of a racemic mixture without the aid of optically active substances.

Since the properties of isomers depend only on the relative position and distance from one another of atomic groups in the molecule, it is clear that molecules, which are mirror-images of one another, must be identical in all those properties which depend on molecular dimensions and attractions. So that their unlikeness can only be expressed in the shape of their crystals, their behaviour to polarised light, or to other asymmetrical forces or substances, such as those in living organisms. This fact was pointed out by Pasteur and by van't Hoff (1901, 2tes Heft, p. 98). On the other hand, isomers will have different chemical and physical properties when their atomic structure is neither the same as, nor the mirror-image of, each other.

Thus, when a compound of a *d*-acid with a *d*-base is compared with that of the same acid with an *l*-base, the two salts are neither of the same structure nor mirror-images, so that they are chemically and physically separable. This is, of course, the usual means adopted for the purpose; the racemic mixture is caused to form salts with an optically active base or acid and it is found that the salt of one isomer has different solubilities from that of the other, so that they can be separated by fractional crystallisation. The lactic acids, for example, can be isolated by combination with the optically active base, brucine. Similarly the two isomeric forms of glucosides, as pointed out above, are not mirror-images and can be separated by crystallisation, etc.

But, if these considerations were invariably and unconditionally true, it would be for ever impossible to separate the components of a racemic mixture without the aid of another optically active compound. Further, unless experiments of producing asymmetric compounds by the action of asymmetric external forces, such as polarised light, are rewarded with more success than hitherto, we are apparently compelled to assume the intervention of unknown, supernatural forces in the origin of life.

Now Emil Erlenmeyer (1913, p. 442) points out that van't Hoff himself shows the possibility of the occurrence of a form of isomerism of a different kind, which may be called *relative*. Thus, when two carbon atoms are united together, there are six free affinities and when these are satisfied by six different univalent groups, twelve different arrangements are possible. But eight of these are derived from the other four by mere rotation, without change of combination. The four different ones are shown diagrammatically in the scheme below, where the two carbon atoms are represented by discs, supposed to be white on one side, black on the other, and the letters, A, B, C, D, E, F, are six different chemical groups.



It will be seen that one or the other carbon atom is supposed to be turned by 180° around the longitudinal axis. See further description by van't Hoff (1901. 2tes Heft, p. 110).

How far all these isomers are capable of existence in a given case is a matter for experiment, but the optical rotations of the four different substances may be understood better if we call the effect of the one carbon atom, A, and that of the other, B. Then we have the four following results:—

$$+A +B, \quad -A +B, \quad +A -B, \quad -A -B.$$

Out of these relative isomers, it is to be presumed that one will be the most stable and that the different relative positions of the various groups will entail different properties. We may take lactic acid and brucine as A and B, for the sake of illustration, so that there are:— *d*-brucine *d*-lactate, *d*-brucine *l*-lactate, *l*-brucine *d*-lactate, and *l*-brucine *l*-lactate.

These facts are of importance in connection with the variety of processes in the living organism. It appears that there is evidence of the existence of malic acids in greater number than usually supposed. Erlenmeyer shows further (p. 447) that, if the *d*- and *l*- forms of a substance are not absolutely fixed "point systems," but convertible by external forces into relative isomers by rotation around an axis of combination, then a force acting in the same direction on the two mirror-images will have a different effect on them, so that they may be converted into modifications which are no longer mere mirror-images of one another, and can therefore be separated by means which do not involve the use of optically active reagents.

The experimental evidence is as follows:—If a solution of *d*-*l*-asparagine (inactive) be boiled for twenty minutes, *l*-asparagine separates out first on cooling. If *d*- and *l*-asparagine are dissolved cold in the same solution, no separation is possible, since their solubilities are identical. One of the two is altered by heating, since the relative solubility is changed. By further fractionation, Erlenmeyer succeeded in preparing specimens of *d*- and *l*-asparagine in nearly pure condition. It appears to be impossible to predict whether *d*- or *l*-asparagine will be obtained in any particular experiment. Further details and also the formation of two different copper salts will be found described in the original paper. The same phenomena were found to exist in the case of the tartrates.

Should these results be confirmed, it is clear that we have a possibility of the production in the plant of optically active substances from racemic mixtures. One of the "relative isomers" would probably be more easily oxidised for energy purposes, leaving in excess the other mirror-image, which had not been changed. Having once obtained asymmetrical compounds, further production is comparatively simple, as we have seen. The method is, however, not a general one. But asparagine itself is of such importance in plants (Horace Brown, 1906) and tartaric acid so common as to suggest that optical activity arose in the way described.

The *Nomenclature of Optical Isomers* is apt to lead to confusion. The actual sign of rotation in related compounds is not necessarily the same. Glucose and fructose belong to the same series, which Fischer calls the *d*-series, although fructose is lævo-rotatory. Onslow has suggested to me that we might use capital letters to express the series, and limit the use of the italics to express the sign of actual rotation. Thus we should say D-glucose, D-fructose, and D-alanine are the related physiologically important members of the same series, although the particular glucose is dextro- and the two others are lævo-rotatory, so that they are also to be called *d*-glucose, *l*-fructose, and *l*-alanine. It appears that this practice might well be adopted.

GROWTH *IN VITRO*

The experiments of Ross Harrison and of Carrel have been referred to previously (page 23). A few remarks with regard to the chemistry of the process are of interest in the present connection. It is plain that in these experiments, granting that new tissue was actually formed, which appears to be satisfactorily shown by the presence of dividing nuclei, the proteins of the blood plasma, used as

the culture medium, must have been utilised for the purpose. In the adult animal the serum proteins are not used as food-stuffs. We have seen that injection of such proteins does not increase the nitrogen output, although that of amino-acids does (Quagliariello, 1912). On the other hand, tissue protein can be used in starvation, so that we must admit the presence of enzymes in the cells able to hydrolyse the proteins. It may well be that, in the growth experiments referred to, these enzymes are called upon to hydrolyse the proteins of the plasma culture medium before they are made use of by the growing tissue.

The presence of autolytic enzymes in tissues can readily be shown by the use of Abderhalden's "silk peptone," a polypeptide containing a large percentage of tyrosine. If a small piece of tissue, say kidney, be immersed in a solution of this substance and kept at 40° the tissue will be covered in a few hours with crystals of tyrosine, from hydrolysis of the polypeptide (Abderhalden und Steinbeck, 1910).

A question cognate to this is the growth of tissue transplanted from one part to another, investigated chiefly by Carrel (1910, 1912), Guthrie, and their co-workers. This has been effected with blood vessels which were removed from the body of the same animal some time previously. But it has been found extremely difficult to transplant the tissues of one animal into another animal, even of the same species. The transplanted tissue usually disappears sooner or later, although, if previously killed by formaldehyde, it seems capable of serving as a support for growth of new tissue on the part of the host.

This fact argues an extraordinary complexity of some kind or other on the part of the tissue protoplasm. The thyroid of one animal, for example, is distinguished by another animal of the same species from its own thyroid. Marshall and Jolly (1907), however, report success in two cases of transplantation of ovaries from one rat to another, apparently remaining functional. Guthrie (1908) also obtained fertile eggs from fowls whose ovaries had been replaced by those of other fowls. Carrel and Guthrie (1906) report a case in which they transplanted the kidneys of a dog into a bitch by vascular anastomosis and then removed the kidneys of the bitch. The transplanted kidneys continued to secrete normally for at least eight days, that is, up to the time at which the paper was written. The urine contained no abnormal constituent with the exception of a trace of protein.

Further facts bearing on the question will be found in Chapter XXIV.

INFLUENCE OF THE NERVOUS SYSTEM ON NUTRITION

At an early period in the history of physiology it was supposed that the only kind of efferent nerves were those causing contraction of muscles. Since the existence of nerves causing stoppage of the heart had been proved by the Webers, and that of nerves producing activity of the cells of secreting glands by Ludwig, it was thought that there were nerve fibres presiding over growth and repair. After section of certain nerves, which, in point of fact, always contained sensory fibres, it was found that the skin, or other sensory surface supplied by them, became inflamed and that wounds on such denervated surfaces did not heal properly. Careful protection of these areas showed that there was no real difference between them and normal areas. The absence of warning on the part of the sensory nerves allowed the infliction of injuries, which would otherwise have been avoided.

Clara Jacobson (1910) made careful experiments on pigeons and on dogs and found that there was no difference whatever between the rate of healing of wounds in normal and in denervated areas.

It is well known that organs grow in proportion to their use, but this is adequately accounted for by the increased blood supply always associated with the activity of any tissue. The manner in which this is ensured will be discussed in Chapter XXIII.

After injury to certain parts of the central nervous system, in fever and after the administration of certain chemical substances, there is a rise of body temperature. Although this effect may be partly accounted for by diminished loss of heat, owing to constriction of the blood vessels of the skin, there appears to be evidence that, in some cases at all events, there is also

an increased production of heat. But it is not necessary to postulate special nerves presiding over this process, since the heat produced in muscular contraction is in itself sufficient to account for the fact. The reader may be reminded of the shivering in fever and in the waking up of animals from hibernation, in which latter process the heat produced by muscular activity serves to bring up the temperature of the animal to its normal height. According to Pembrey (1903) a dormouse raises its temperature by 19° in forty-two minutes.

Cathcart and Leathes (1907) found the amount of uric acid excreted to be different according to the manner in which the muscular contraction was produced. If shivering was brought on by exposure to cold, a marked rise in uric acid output resulted; whereas, if vigorous voluntary contraction was produced, there was a marked *fall* in the output. Although these experiments do not prove the existence of nerves directly affecting chemical changes, they suggest that different kinds of innervation produce different chemical reactions in the cells affected.

When cells are known to be supplied with nerve fibres, it would be rash to deny the possibility of the cell processes being influenced by impulses passing from the fibres to the cells. All that we are justified in saying is that there is, as yet,

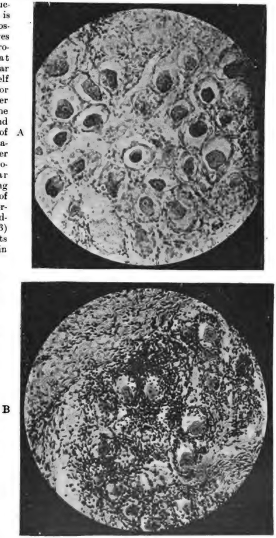


Fig. 77. Photographs of histological changes in the posterior root ganglion in a case of herpes zoster of the left side of the neck.

A, Normal third cervical ganglion of the *right* side.

B, One of the inflammatory foci in the third cervical ganglion of the *left* side.

Both ganglia were cut in the same block, fixed on the same slide, and stained together by Nissl's method.

Note the multitude of small dark cells in *B*, and the effects on the ganglion cells.

(Head and Campbell, 1900, Pl. 4.)

no satisfactory evidence that the nutrition of the tissues is directly affected thereby.

On the other hand, the work of Head and Campbell (1900) on *Herpes Zoster* requires consideration. This disease results in the formation of blisters on the skin in the area of distribution of particular nerves. It was shown by the investigators named that these changes in the skin are caused by irritative changes in the dorsal root ganglia (see Fig. 77). Owing to these changes, abnormal impulses are sent in an efferent direction along the sensory fibres to the skin. Although I have been able to show (1901, 2) that dilatation of blood vessels in the skin is produced by excitation of the sensory fibres of dorsal roots, it seems difficult to believe that mere vascular dilatation should cause the actual formation of blisters. At the same time, the possibility has not been disproved.

MATHEMATICAL LAWS OF GROWTH AND OF METABOLISM

It might be supposed that such complex processes as those discussed in the present chapter would be impossible of attack on mathematical lines.

Slator (1913) has shown that the growth of yeast can be expressed by a logarithmic formula. If a culture medium be inoculated with N cells of yeast per cubic centimetre, the rate of growth at a given moment of time is proportional to the number of cells present at that time, that is $N + n$, where n is the increase in number during the time which has elapsed since the inoculation. This is clearly a case of the "compound interest" law, which was explained on page 36 above, and the simplest assumption that can be made is that the increase in number is in direct linear proportion to $N + n$, that is:—

$$\frac{dN}{dt} = K (N + n),$$

where K is some numerical constant. On integration, this equation becomes:—

$$K = \frac{1}{t} \log_e \frac{N + n}{N}.$$

It might be supposed, not unnaturally, that this simplest formula would be found to be insufficient, but Slator has shown by four different methods that it does actually express the results until nearly the end of the period of growth, when food supply is restricted and metabolic products inhibit growth.

The four methods used were:—(1) counting the cells directly, (2) the rate of fermentation by measurement of the rate of formation of carbon dioxide, (3) the growth constant, K, is estimated by counting the number of cells and the rate of fermentation while the time is eliminated, and (4) by comparing the time taken for two cultures, inoculated in a known ratio, to arrive at some definite stage. In malt extract, with a small amount of hops, it was found that the time taken to double the number of cells was 2·9 hours (see also the work of Horace Brown, 1914). The growth of bacteria is treated by Slator (1916 and 1917).

Even in the metabolic processes of the higher animals, we have already seen reason to regard the operation of the law of mass action as being uninterfered with, more especially where we know the reaction to be reversible. An interesting instance of rate of reaction being proportional to the concentration of the substances reacting is to be found in a paper by Hoesslin and Lesser (1911, p. 356). If a fasting dog is fed with meat, the nitrogen excretion is 14 to 15 per cent. greater if it is given all at once, than if the same total amount is given in six separate doses, at intervals of three to four hours.

Osborne and Mendel (1915) show that rats, on inadequate diet, may remain, for a time much longer than their period of growth, at a small size. When given at any time a complete diet, they grow to their normal size, but not beyond.

The Mendelian Laws of Heredity will be referred to presently.

PHYSIOLOGICAL PROCESSES OF THE LOWER ORGANISMS

The fundamental processes of life in all organisms are, no doubt, similar, so that it appears to be held by certain investigators that, on account of the

comparative simplicity of structure of the "lower" organisms, we are more likely to be able to discover what is the essential nature of these processes, if we devote our attention to the apparently simpler creatures.

Without denying the great value of the comparative method in eliminating merely incidental phenomena, it must be pointed out that this very simplicity is, in the majority of cases, a disadvantage. The same organ, or even cell, fulfils a variety of purposes, which in the higher organisms are relegated to distinct groups of cells. Moreover, the size of the organism is of much importance, as will have been sufficiently obvious in the present chapter. The science of nutrition would be almost impossible without the larger, warm-blooded animals. The advantage of the increased rate of reactions, owing to the higher temperature, is not to be undervalued.

The physiology of unicellular organisms, although of considerable importance in special aspects, is not to be regarded as a "general physiology." Indeed, if the choice had to be made between the investigation of simple or complex organisms alone, there is no doubt that a much more general and fundamental body of doctrine would be obtained from the latter.

The following remarks of Claude Bernard (1866, p. 100) may be read with interest: "Il ne faudrait pas croire, en effet, que l'animal inférieur est plus simple ou que ses fonctions sont moins compliquées ou moins nombreuses; et qu'on pourrait les prendre pour ainsi dire à leur naissance, pour suivre ensuite leur développement dans les animaux supérieurs, qui auraient ainsi des propriétés nouvelles se surajoutant aux premières. L'animal inférieur possède toutes les propriétés essentielles qu'on retrouve aux degrés les plus élevés de l'échelle des êtres; mais il les possède à l'état confus, et pour ainsi dire répandues dans toutes les parties du corps. Ainsi l'infusoire, qui s'agite et se dirige dans le liquide où il a pris naissance, possède évidemment la propriété de se mouvoir; il doit être doué de sensibilité pour déterminer ses mouvements; enfin, il peut se reproduire, puisque l'espèce ne périt pas. Voilà donc la vie à son degré le plus infime, avec toutes les fonctions qu'elle manifeste chez les animaux élevés. Mais quand on cherche les organes de chacune de ces fonctions, on ne peut plus rien distinguer, et c'est à ce point de vue seulement qu'on doit parler de la prétendue simplicité des animaux inférieurs."

REPRODUCTION

In the most primitive state it frequently happens that the whole cell contents of an organism divide into a number of smaller parts, each of which gives rise to a new organism. In such a process the new organisms are endowed only with the qualities of the one cell. Since the powers of adaptation of any two organisms or cells are not, as a rule, identical, it is clear that if the new organisms could "inherit" the characteristics of more than one it would be to its advantage. Accordingly, we find, very early in the course of evolution, arrangements by which two cells join their forces by fusion or conjugation. At first, the two cells are similar, as in Spirogyra, but almost at once we find a differentiation by which a large cell, called the female cell or gamete, is incapable of further development without fusion with another smaller, usually motile, cell, the male gamete. The great variety of arrangements by which this "fertilisation" is effected or facilitated are beyond the scope of this book and will be found in the textbooks of botany and zoology. The main point to be kept in mind is the incapability of either the male or female cell alone to grow to a new organism. Since it is the female cell which remains more or less stationary and is, as it were, sought out by the male cell, while the new organism grows from the fertilised female cell, the obvious effect of the male cell is to set into activity the dormant powers of segmentation and growth of the female cell. It is easy thus to lose sight of the fact that the male cell also brings with it the capacities of the organism from which it has arisen.

The mysterious power of the male has from the earliest times excited wonder and has, not unnaturally, become the object of religious worship. It is indeed greatly to be regretted that the sexual process should have become the subject of unseemly jesting. Of course, incidents of real humour may arise in any connection, without detriment to its essential solemnity, as witness the great art of Shakespeare. But I feel compelled to state my belief that much mischief is done by the habit of looking upon anything related to sex as, in itself, a

matter for jesting, apart from any real humour. Possibly, the excessive secrecy and reticence maintained on the question are much to blame, and there is no doubt that the wider teaching of a proper physiology in schools will have a good effect in this direction. The almost universal ignorance of matters of the most vital importance to the community, as well as to the individual, is scarcely less than amazing. It is much to be hoped that in the future the sexual process will be looked upon as something essentially beautiful and good, in fact as καλός in the old Greek sense. Consider the glorious manifestation of sex in the "lilies of the field" and how the love of man and woman has been the motive force of many of the greatest and noblest deeds in the world's history.

Owing to the very urgency of the impulse for the sake of the preservation of the race, charitable excuse may be made for those who offend; but condemnation must be unsparingly given to those who tempt others to sin.

The view taken by Sir Thomas Browne ("Religio Medici," vol. i., p. 100 of Sayle's edition, 1904) is much to be regretted. On the other hand, Geddes and Thomson's "Sex" (1914) takes the true and noble position.

After this apparent digression, for which I offer no apology, we may continue what may perhaps be regarded as the proper subject of our book. In reference to the, as yet, mysterious power of the male cell to excite the process of development in the female cell, the work of Loeb (1900) should be mentioned. This investigator showed that "*artificial parthenogenesis*" can be produced to a certain extent by treatment of the eggs of sea urchins in various ways.

The following is the most effective of these. The eggs are first placed for 1·5 to three minutes in a mixture of 50 c.c. of sea water with 2·8 c.c. of 0·1 molar butyric acid. They are then removed to 200 c.c. of sea water. Fertilisation membranes are formed, but nothing more happens. The next step after the eggs have remained for twenty minutes or more in the natural sea water is to remove them to hypertonic sea water, made by adding 8 c.c. of 2·5 molar sodium chloride to 50 c.c. of sea water. The actual time they require to remain in this solution can only be found by trial, so that samples are withdrawn every five minutes, after they have remained for fifteen minutes. This taking of samples is continued for sixty minutes. Those that have remained for the correct time develop into normal larvæ on removal to natural sea water. Further details may be found in the book by Loeb (1909).

In rare instances, as the well-known one of the bee, where unfertilised eggs develop into drones, natural parthenogenesis is to be met with. It will be obvious, however, that the advantages of mixing the qualities of two individuals is absent in all these cases of parthenogenesis. What we learn from the experiments of Loeb is that it is only under a combination of chemical and physical influences, such as is very unlikely to occur in natural conditions, that the female cell, except in such rare cases as that of the bee, is able to develop without the co-operation of the male cell. In this way the advantage of sexual reproduction, the union of two individuals, is ensured. At the same time, we see that the female cell actually does possess the power of development apart from the entrance of the male cell.

The work of Przibram on "Embryogeny" (1908) may be consulted for the laws governing development.

MENDELISM

The facts of heredity have, of recent years, become more or less amenable to scientific treatment, mainly by the work arising from that of Mendel, abbot of Brünn. This work was published in 1865, but did not become known until its discovery by De Vries in 1900.

It is only possible in the limits of space permissible here to give but the merest outline of the fundamental facts. The reader is referred for further details to the book by Bateson (1913).

In order to be able to follow the process of inheritance from generation to generation, Mendel directed his attention to some single character; in the Sweet Pea, for example, he took the quality of tallness and dwarfness. Suppose that a tall individual was crossed with a dwarf one, it was found that the next generation consisted entirely of tall individuals. The quality of tallness was called, therefore, "*dominant*," while that of dwarfness was called "*recessive*," since

it was found to be merely dormant, as it were, because it reappeared in the next following generation. The tall individuals of the first generation were allowed to fertilise amongst themselves only and, in the whole number of individuals produced, there was found to be one dwarf to every three talls. The remarkable fact is that the dwarfs are found to be of pure breed, that is, all of their posterity are dwarfs. Of the talls, one-third are pure breed, the other two-thirds will give the same mixture of one dwarf to three talls in the next generation and so on. We shall see presently how this proportionality is to be explained. For the present, we note that, although they may be the offspring of two tall individuals, the dwarfs are pure breed (Fig. 78, from Bateson's Address, 1906, illustrates some of these facts).

FIG. 78. PHOTOGRAPH OF SWEET PEA PLANTS.—Five tall and three dwarf, grown from the same pod from a cross between a tall and a dwarf variety. The dwarf plants on the left are pure breed, recessive, although one of the parents was tall. The tall character is the dominant one, and some of the tall individuals are mixed breed. The whole family corresponds to the stage F_2 of Fig. 79.

(Bateson, 1906, Pl. 1.)

Now, in the sexual cells, or gametes, male and female, the chromosomes, arising from the nucleus by mitotic division, are only half the number of those formed in the somatic or general body cells; so that, when the sexual cells unite to form a zygote, the normal number is again attained. Taking, for simplicity, the characters of blackness and whiteness whose heredity is to be traced, the scheme of Fig. 79 will represent matters.

It is to be remembered that blackness and whiteness do not always obey simple Mendelian laws, so that any character taken at haphazard cannot be used to trace the laws of heredity.

We have just seen that, compared with the germ cells, the body cells are double structures, so that, for the present purpose, we may represent a pure black individual by two black symbols, say rectangles, each rectangle representing a germ cell. A pure white individual is represented by two white rectangles. Further, when a black and white individual are crossed, the hybrid contains

both characters, but, when it forms germ cells, the qualities are separated again, so that each germ is either black or white, not a mixture of the two, and an equal number of black and white cells are formed. When one of the characters is dominant, that is, when it is such as to overpower the manifestation of the other, which is recessive (in our case, let us call blackness, dominant, and whiteness, recessive), the result will be as in the diagram of Fig. 79 (Bateson, 1906). The gametes are represented by the single letters and rectangles, the zygotes by each pair of these. To show that blackness is dominant, in the zygotes the black rectangles are placed on the top of the white ones. The possible combinations are as shown and, since black is dominant, individuals composed of black and white will appear to be black and indistinguishable from those composed of all black. In this way, as can easily be seen, there will be three black to one white, or three dominants to one recessive. In reality, two of the three dominants are impure. If one of these, DR, is crossed with RR, an equal number of blacks and whites will result (see the second diagram of Fig. 79); while, if DR is crossed with DD, all blacks will appear (third diagram). We see also that when, in fertilisation, a white, recessive germ meets another white germ, the result is pure white, and blackness is thrown out from that family altogether. Similarly for the pure black germs.

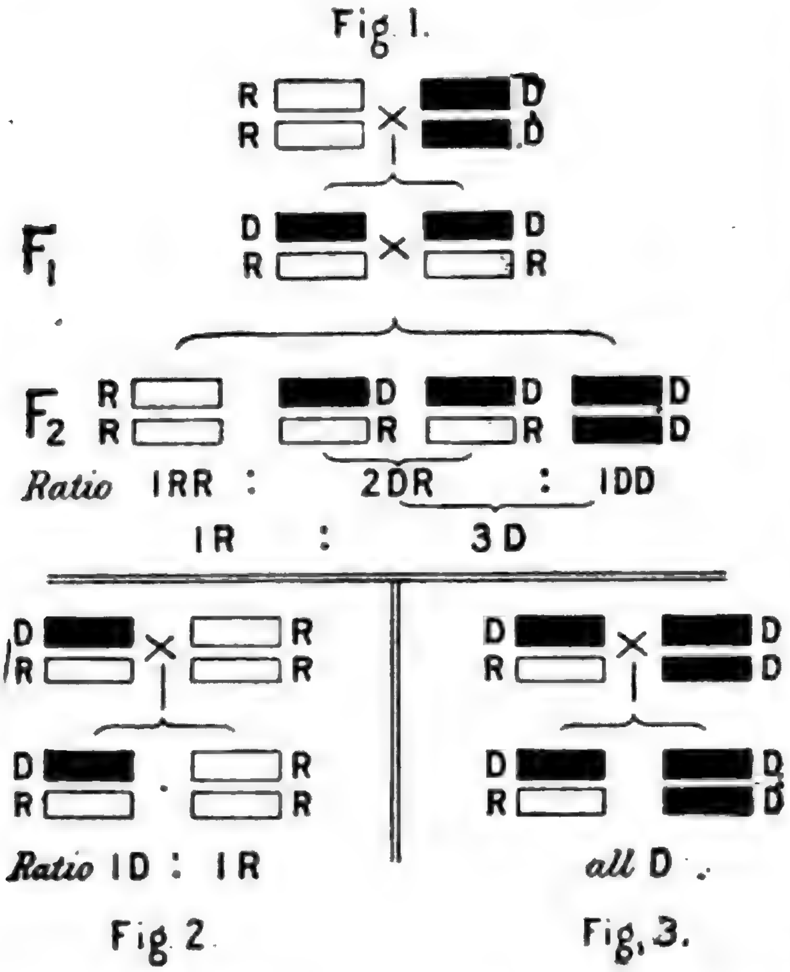


Fig. 79. Bateson's diagram of segregation of dominant and recessive characters.

(Bateson, 1906, p. 159.)

It follows that, when we know that a particular character is dominant and another is recessive, we can say with confidence that when a recessive individual has once made its appearance, all its descendants will be recessive, assuming, of course, that it is not crossed with a dominant individual. For example, the original Chinese primrose has a palmate leaf. About 1860 a variety appeared with a pinnate leaf. The first is dominant, the second recessive, so that whenever the latter appears it continues to breed true.

Naturally, the matter is not always quite so simple as this, even when we have found out which character is the dominant. There are interactions, in some cases, between the factors, and the manner in which, say, a colour is produced has to be taken into account. Colour is not necessarily a single character, and the factors which produce it are *separately* transmitted, giving rise to the variety of colours met with, especially in flowers. The complex inter-relationships, nevertheless, are doubtless capable of resolution.

As an illustration, the analysis by H. Onslow (1915) of the difference between recessive whiteness, or albinism, and dominant whiteness may be mentioned.

Another interesting case is when the same character, as that of bearing horns in sheep, may be dominant in the one sex, recessive in the other. The case of horned and hornless sheep was investigated by T. B. Wood (1905).

This very short account may serve to indicate the kind of problems that may be attacked from the Mendelian point of view.

SYMBIOSIS

In certain unicellular animals, such as Euglena, we find chlorophyll grains, as if in a plant cell. Some species of multicellular animals, also, such as *Hydra viridis*, and the planarian worm, *Convoluta Roscoffensis*, take in algæ and carry on a partnership in nutrition, as it were. Keeble (1910) shows that this Convoluta, after it has taken up the green algæ, is able to live and grow in sea water which contains no solid particles, and only traces of organic matter of any kind. The algæ are able to decompose carbon dioxide and form carbohydrate, as under normal conditions. It appears that the starch, thus formed, is changed into fatty substances by the vegetable cells and passed on to the animal cells in this form. Starch itself cannot be attacked by the cells of the animal. Rows of fatty particles are seen apparently passing from the green cells to the neighbouring animal cells. It is, of course, possible that sugar also may pass, as such, to the latter cells.

Without the green cells, *Convoluta Roscoffensis* fails to grow, and the same thing happens in darkness. At a certain stage of its existence, the organism ceases to take in solid food and depends entirely on its vegetable partners.

Keeble has shown further (1910, p. 123) that the infecting algæ are capable of independent existence.

An interesting question is why the infecting algæ grow so rapidly as they do inside the animal organism. It is obvious that they must obtain nitrogen and it is very significant that *Convoluta Roscoffensis* and *C. paradoxa*, which also contains symbiotic algæ, are peculiar amongst the Turbellarian worms in possessing no excretory system for the waste products of their nitrogenous metabolism. The conclusion is clear; these waste products are utilised by the algæ. In fact, it is actually found that these particular algæ grow better when supplied with their nitrogen as uric acid than as nitrate. There is, moreover, evidence that, not only do the cells of the algæ supply the animal cells with fat and carbohydrate, but also with nitrogenous food, which they are able to hand on after having converted that obtained from the sea water into a form with which animal cells can deal.

The reader is recommended to consult the fascinating little manual by Keeble (1910) for further information.

SUMMARY

The use of food in the growing organism is to supply material for construction of body substance, to replace that lost in wear and tear, and to give energy for the performance of muscular movements as well as for the bringing about of endothermic reactions. In the adult, of course, the necessity of a supply for growth is absent.

The amount required for the replacement of wear and tear, or maintenance, is small.

In growth, it is obvious that all the chemical elements which are constituents of the organism must be supplied in some form or other, but, while very simple chemical compounds suffice for the lower organisms, the capability of dealing with such is, to a large extent, lost by the animal, even at a comparatively early stage of the protozoa. These require food in the form of complex organic compounds already prepared by other animals or plants. As carbon, nothing less complex than glucose; as nitrogen, nothing less complex than amino-acids suffice for these animal organisms.

In addition to the known organic compounds, the presence of traces of some substances, whose constitution is as yet unknown, is necessary, not only for growth, but also for maintenance. It appears, however, that there are some of these which are absolutely indispensable for growth, but unnecessary for maintenance. These "accessory factors" do not act by forming part of the constitution of definite chemical compounds such as the proteins of the protoplasm, but as "hormones" or catalysts; although the possibility of their forming some essential part of the cell mechanism, such as the surface membrane, has not yet been definitely excluded.

For the purpose of energy production, the supply of due amounts of carbon and hydrogen alone is, in theory, sufficient. Nitrogen is absolutely necessary merely for the purpose of replacing that which is lost from the structure of the machine in wear and tear. At the same time, there appear to be certain advantages in taking a larger proportion of nitrogen food, at all events, for most people. The protein content of most standard dietaries is certainly, however, unnecessarily high.

There are some organisms which have very special requirements as to food materials.

As naturally follows from the facts given in preceding chapters, the presence of inorganic salts in food is essential.

The method by which atmospheric nitrogen is made available for the food of plants and animals is described in the text. The stages are, briefly, bacteria of the soil, and in the root-nodules of leguminous plants, proteins of plants and animals, ammonia, nitrites, nitrates. The latter are again utilised by plants, part being lost as nitrogen gas, and if not used up, by a reverse process back to ammonia.

With regard to the "accessory factors," or hormones, it will be clear that, if a certain chemical grouping is required for a special purpose in the organism, such as an internal secretion, and if the organism is unable to synthesise it for itself, then it must be given in the diet. Such a substance is tryptophane for the growing rat. But there is something else needful. Rats will not grow on a diet of pure protein, fat, carbohydrate, and salts, even when containing all the known chemical constituents of food, including tryptophane. There is a substance present in a minute amount of milk, or boiled extracts of fresh vegetables or meat, which is absolutely necessary. Moreover, when these factors are present, animals are able to preserve their tissue nitrogen on pure amino-acids.

Certain diseases, such as beri-beri and scurvy, have been shown to be caused by the absence of similar "accessory factors" in diet.

There is some evidence that even yeast and bacteria are dependent for growth on similar "accessory factors."

The chemical constitution of proteins, as condensations of amino-acids of different kinds, is described briefly in the text. These substances, when taken as food, are first hydrolysed in the organism, by means of the digestive enzymes, to their constituent amino-acids and related substances. The greater part of these amino-acids, passing into the blood, is de-aminated, mainly, or perhaps exclusively, in the liver; the resulting ammonia is converted to urea, again mainly, or perhaps exclusively, in the liver; the hydroxy or ketonic fatty acids produced are burnt up for energy purposes. The small part of the amino-acids not de-aminated is used by the tissues for growth or for replacement of loss by wear and tear.

There are, then, two more or less distinct forms of protein metabolism, one for energy purposes, "*exogenous*," in which the nitrogen is lost, the other for replacement of loss or for growth, "*endogenous*," in which the nitrogen is retained.

The question of the minimum nitrogen requirement is discussed in the text. The amount absolutely necessary for a healthy man, doing the ordinary amount of work, has been reduced to 3·5 g. per day, equivalent to 21 g. of protein. The total energy value of the diet, expressed in heat units, must not be less than 3,000 calories, made up with carbohydrate and fat.

There is evidence that the presence of carbohydrate is essential for the synthesis of protein, both in the animal and in the plant.

In the wear and tear of the protoplasmic mechanism only a certain part requires replacement, not the whole of a complex molecule.

The importance of creatine and purine metabolism is pointed out.

In muscular work, so long as it is not excessive, no evidence of increased nitrogen excretion, due to wear and tear, or otherwise, is to be obtained. It appears that a complex substance, containing nitrogen and of a high energy content, is broken down to give the energy of the contraction process. The nitrogenous constituent is normally used again for resynthesis of the "inogen," while the carbon and hydrogen are burnt up.

The chief function of carbohydrate food, as also of fat, is to afford energy; but, in the process of its oxidation, a number of intermediate products are produced, given in the form of a diagram in the text (page 273). These substances are of importance in that they give opportunity for the occurrence of reactions of importance to the organism in other ways. Pyruvic aldehyde, lactic, and pyruvic acids may be especially mentioned. All of these reactions, with the exception of the last stages of oxidation, have been shown to be reversible under conditions obtaining in the living organism.

Fat is of additional importance as being readily stored in considerable quantity. It can be formed from the carbohydrates of the food, and the manner in which there is every reason to suppose that the process takes place is, in general terms, as follows. By condensation of an aldehyde with a ketonic acid, we obtain another aldehyde with two more carbon atoms than the original one and, by repetition of the process, with final reduction, fatty acids with straight chains of carbon atoms, increasing by two at a time, are produced.

The frequent occurrence of pyruvic acid in the processes of metabolism, of carbohydrate, fat and protein, is pointed out. This substance forms, as it were, a meeting place of the three different classes of food-stuffs.

The value of perfusion experiments and experiments *in vitro* as extended to processes in the whole organism is discussed in the text.

Carbohydrates and fats are readily stored in the tissues as glycogen and neutral fats, respectively. There does not seem to be any particular form in which protein is stored, except as tissue or protoplasmic substance, although amino-acids can be adsorbed by tissue colloids.

The effect of ammonia and of urea in diminishing the nitrogen loss is probably due to a diminution by mass action of the de-amination of amino-acids and of the formation of urea from ammonia.

The question of optical activity is discussed in the text and the way in which compounds of this kind may have first arisen is described. The preferential use of one optical isomer, at all events for energy purposes, is shown to be merely one of degree, although the cell constituents are finally composed of one set of isomers.

Results obtained by the growth of tissues *in vitro* show that proteins can be utilised, or dealt with in some way, by cells themselves, a fact also evident from the using up of cell substance in starvation. The process is to be explained by the presence of autolytic enzymes. Under normal conditions, the proteins of the blood do not serve as nitrogen food to the cells of the tissues.

The fact that an organ of one animal has not been satisfactorily transplanted into another one, apart from exceptional cases, argues an extraordinary complexity of some kind or other on the part of the protoplasmic systems of the cell.

There is no evidence that the processes of nutrition in cells are directly influenced by the nervous system; although the existence of nerve fibres supplying cells forbids a categorical denial of the possibility of such influence.

Certain processes of growth and metabolism obey definite known mathematical laws.

It is pointed out that the investigation of functions of the lower organisms is less likely to lead to valuable knowledge than that of the higher organisms. The methods of comparative physiology are of value in enabling us to exclude unessential factors and, in certain cases, allow experiments to be made under conditions in which it would be impossible to preserve the organs of warm-blooded

animals in a normal state. The fundamental phenomena of general physiology cannot be discovered by confining our attention to the lower organisms.

The essential fact in the physiology of sexual reproduction is the advantage gained by the union of the capacities and qualities of two cells from different individuals. Special cells are set apart for this purpose, each being incomplete and incapable of development without the concurrence of the cell of the opposite sex. In the case of the female cell, this incapacity of development is to a certain degree only a relative one. The eggs of some invertebrates can be made to develop by chemical agency and a few rare cases are known where the unfertilised eggs develop into adult animals.

A short account is given of the facts of heredity as treated on the principles laid down by Mendel.

In certain cases, plant and animal cells live side by side in the same organism (symbiosis). The plant cells contain chlorophyll and afford carbohydrate material for the animal; while the cells of the latter appear to provide nitrogenous food for the growth of the plant.

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CHAPTER X

CATALYSIS AND ENZYMES

FUNDAMENTAL FACTS

SINCE the work of Berthelot and Péan de St Gilles (1862) it has been a familiar fact that, if ethyl acetate and water be mixed in molecular proportions, and allowed to remain for some weeks, the ester is hydrolysed with formation of ethyl alcohol and water; but, however long a time be allowed to elapse, only a certain part of the ester undergoes conversion, although there is sufficient water to hydrolyse the whole. The rate of change becomes slower and slower until it ceases, and at this time it is found that the four components of the system are present in the proportion of one-third of a gram-molecule each of alcohol and acid, two-thirds of a gram-molecule each of ester and water.

Further, suppose that we have commenced with acetic acid and alcohol, also in molecular proportion, and have allowed the reaction to proceed until it stops, we find that we obtain the same proportion of the four components. Clearly we have to deal with a case of equilibrium, or balance of opposite reactions.

Now these reactions, which for the present purpose we may regard as being spontaneous, are extremely slow. We can, however, increase their rate enormously by adding some mineral acid. In this case, the attainment of equilibrium, which, left to itself, takes weeks, can be brought about in a few hours. There are three important facts to be noticed here. Firstly, the composition of the system in equilibrium is the same under the action of acid as when reached spontaneously. Secondly, the acid added is found, after its work is done, still present in the same state as it was originally. Lastly, whether we start from ester and water, or acid and alcohol, we find that the rate of the reaction is accelerated by the addition of acid. This latter fact follows, as we shall see later, from the other fact that the equilibrium position is not changed by the presence of the acid.

Let us take now, instead of acid, an extract of the pancreas and, in place of ethyl acetate, another similar ester, amyl butyrate.

The experiment was made by Dietz (1907) and the higher ester was used for convenience in calculating the results, since the spontaneous reaction is so slow as to be undetectable during the time of the experiment; in other respects, the system may be regarded as precisely similar to the previous one.

The effect of the pancreatic extract is even more powerful than that of acid in accelerating the reaction. Otherwise, the three facts to which attention was called in that case are also to be noticed in this, with one slight exception, namely, that the position of equilibrium is not quite the same as that under acid or the spontaneous one. Fig. 5 (p. 92) in my monograph on "Enzyme Action" shows that the position of equilibrium is the same when attained from either direction, a fact also obvious from Fig. 80 of the present work.

One more case will be instructive, before we proceed to discuss the meaning of the facts before us. This is the one to which Fig. 81 refers. The system here is one of glucose, glycerol, glycerol-glucoside, and water. The equilibrium is brought about under the agency of a substance obtained from almonds, and known as emulsin. The curves, taken from experiments of my own (1913), show that the equilibrium position is the same, whether we start from glucose and glycerol or from glucoside and water. The additional fact is that we are dealing with

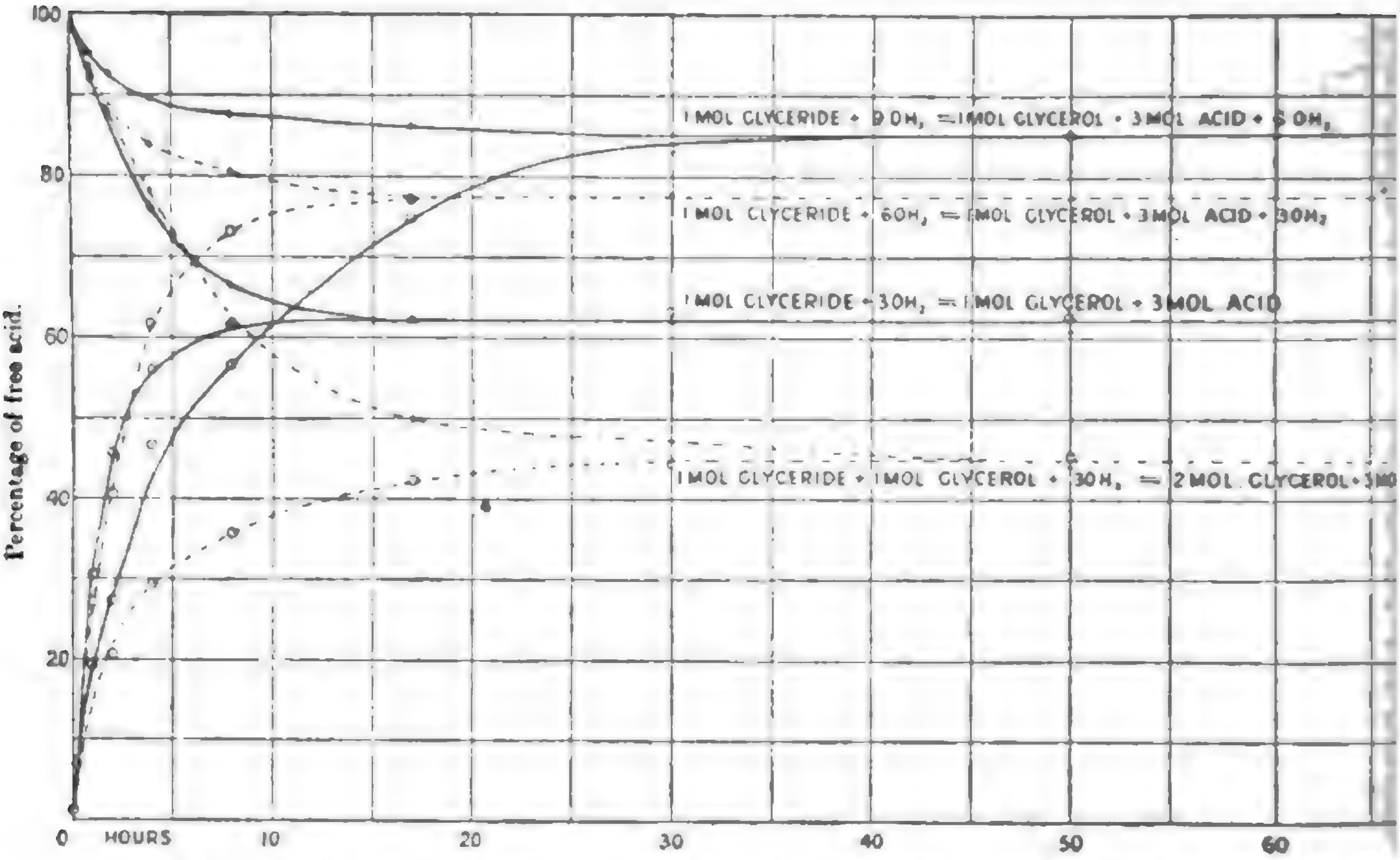


FIG. 80. SERIES OF CURVES SHOWING THE DIFFERENT EQUILIBRIUM POSITIONS OF THE OLEIC ACID-GLYCEROL FAT-WATER SYSTEM, AS ATTAINED UNDER THE ACTION OF LIPASE WITH DIFFERENT PROPORTIONS OF WATER.

Note that the greater the concentration of water, the nearer is the equilibrium point to that of complete hydrolysis (upper three pairs of curves).

The presence of excess of glycerol (lowest pair of curves) leads to increase of synthesis, by removal of water as well as by mass action.

Ordinates—percentage of free acid.
Abscissæ—time in hours.

(Armstrong and Gosney, 1914, p. 183.)

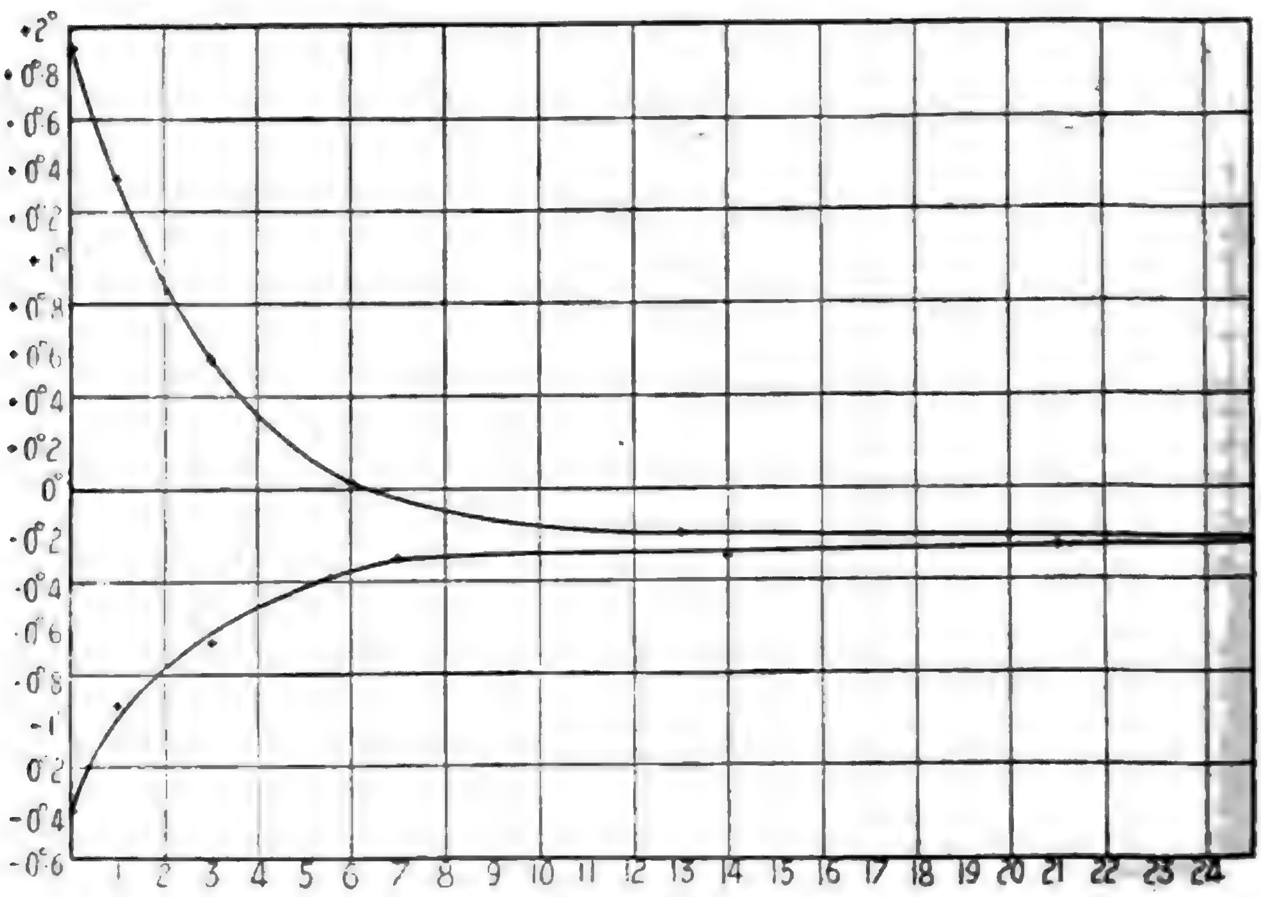
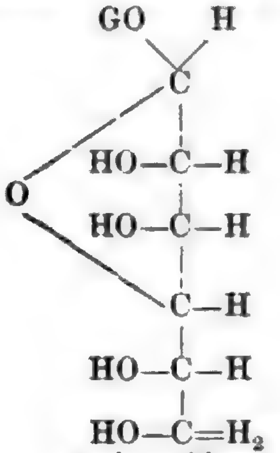


FIG. 81. EQUILIBRIUM ATTAINED UNDER THE ACTION OF EMULSIN, ON GLYCEROL AND GLUCOSE (UPPER CURVE), ON GLYCEROL-GLUCOSIDE (LOWER CURVE).—The position is the same.

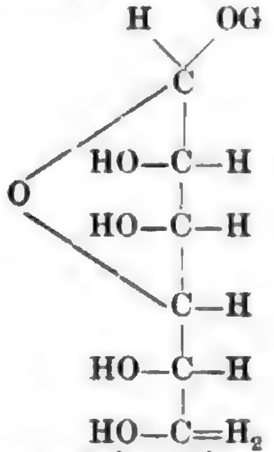
Ordinates—optical rotation of diluted samples.
Abscissæ—time in days.

(Bayliss, 1913, 1, p. 242.)

optically active substances. Glucose is dextro-rotatory and we find that, as glucoside is formed, the rotation of the mixture diminishes, finally passing to the lævo-side of zero. Now there are two possible optical isomers of the glucoside, according to the position of the glyceryl group in relation to the terminal hydrogen atom of the glucose. Thus, diagrammatically, putting G for glyceryl :—



α-glucoside.



β-glucoside.

The one on the left, called for convenience the α-glucoside, has a higher dextro-rotation than glucose, while the β-glucoside is lævo-rotatory. The preparation from almonds, which was used in the experiments, is found to cause hydrolysis of the series of β-glucosides only, of which there are a great number. The experiment quoted shows that it also brings about synthesis of the β- form of the glycerol-glucoside, since that one formed is lævo-rotatory. Similar results were obtained by Bourquelot and Bridel (1913) in the case of numerous glucosides of alcohols. Note that the two glucosides are not mirror-images (see page 284 above).

An important fact, which will be found to be of much significance in later pages, is that the reaction takes place in alcohol of such a strength that the agent, emulsin, is completely insoluble in it and can be filtered off, leaving no trace in solution. The same statement applies to the experiment of Dietz with extract of pancreas, or lipase, as the active constituent has been called.

CATALYSIS

What is the meaning of all these facts?

To begin with, it will have been plain from the facts given in the chapter on Nutrition that the chemical changes which take place in the living organism are of a kind such as, in the laboratory, can only be brought about by powerful reagents and high temperatures. Take the hydrolysis of protein to amino-acids. This is effected in the laboratory by boiling concentrated hydrochloric acid, but in the organism it takes place, at an equal rate, at ordinary temperatures and in a medium which is only just faintly alkaline or neutral. This fact especially attracted the notice of Schönbein (1863).

Berzelius (1837, pp. 19-25), however, directed the attention of chemists to what he called a "force which differs from those hitherto known." On account of the importance of the question, I will give, in an abbreviated form, the description given by Berzelius, whose portrait is reproduced in Fig. 82.

The difficulty to which attention has just been called, is pointed out by this chemist. Blood is supplied to an organ and, without the assistance of any other liquid, we obtain saliva, milk, urine, and so on. A discovery was made by Kirchhof (1812) which gave the first clue to an understanding of the vital processes, but which, as it is scarcely necessary to remark, are still far from complete explanation. Kirchhof found that starch could be converted into glucose by the action of dilute sulphuric acid, which was itself unchanged in the process, since it could be recovered at the end. The next step was, according to Berzelius, the discovery of hydrogen peroxide by Thénard. This was noticed to be decomposed, not only by soluble alkalies, but also by many various kinds of solid insoluble substances, such as manganese peroxide, silver, platinum, and the fibrin of blood. These do not take part themselves in the new

compounds formed, but remain unaltered; they are stated to act by an "indwelling force, whose nature is still unknown." Shortly before Thénard's discovery, Humphrey Davy had found that platinum, under certain conditions, had the power of causing the oxidation of alcohol vapour in air. Edmund Davy, his cousin, made a more active preparation, which was actually platinum in a very finely divided state, and Döbereiner made a spongy platinum which could even cause the union of oxygen and hydrogen gases. As we shall see later, this greater activity is due to the greater extent of surface. Berzelius points out that this property is not confined to platinum, but, in a less degree, is possessed by other substances. Thus, while platinum is active even below 0° C., gold requires a higher temperature, silver still higher, and glass at least 300°. He next refers to the phenomena of alcoholic fermentation, known since the earliest times, before written history. But, until Cagniard de Latour (1838), the fact that it was produced by a living organism was unknown. The words used by Berzelius are worth quoting: "We had made acquaintance with the fact that, for example, the change of sugar into carbonic acid and alcohol takes place in fermentation under the influence of an insoluble body, which we call 'ferment,' and also with the fact that this ferment could be replaced, although less effectively, by animal fibrin, coagulated plant albumin, cheese and similar substances, as well as with the experience that the process could not be explained by a chemical action between the sugar and the ferment analogous to double decomposition. Comparing it with known relations in the inorganic world, it was seen to be most like the decomposition of hydrogen peroxide under the influence of platinum, silver, or fibrin; it was, therefore, natural to suppose that the action of the ferment was an analogous one." The investigations of Mitscherlich on the formation of ether from alcohol by sulphuric acid are next brought into connection with those of Kirchhof on sugar and with the action of alkalies in decomposing hydrogen peroxide. What is common to all is the manifestation of a "new force," different from chemical affinity in the ordinary meaning of the words,

Jac. Berzelius

FIG. 82. PORTRAIT OF BERZELIUS.

(From the painting by Södermark.)

in that a substance may effect chemical changes without itself taking part in them. Berzelius is careful, however, to guard himself from the supposition that this force is other than a special manifestation of known properties of matter. We shall see later that, in certain cases, explanation on the lines of known chemical and physical laws is actually possible. To return to our author, we find a definition of the process given as follows: "I will call it the *catalytic* power of substances and the decomposition effected thereby, *catalysis*; just as we understand by *analysis* the separation of the constituents of substances by means of ordinary chemical affinity. Catalytic power appears to consist essentially in the fact that substances are able to set into activity affinities which are dormant at this particular temperature, and this, not by their own affinity, but by their presence alone."

Turning to living nature, it is pointed out that "we have justifiable reasons to suppose that, in living plants and animals, thousands of catalytic processes take place between the tissues and the liquids and result in the formation of the great number of dissimilar chemical compounds, for whose formation out of the common raw material, plant juice or blood, no probable cause could be assigned. The cause will perhaps in the future be discovered in the catalytic power of the organic tissues of which the organs of the living body consist."

With respect to the name itself, it must be admitted that "catalysis" suggests an opposite kind of process to that of "analysis"; so that, since this latter implies the separation of a process or compound into its constituents, catalysis might be taken to mean a synthetic process. The word has come into general use, however, to denote such processes as those referred to by Berzelius. It is also convenient to have a word for the agent itself: "catalyst" is most frequently used, sometimes "catalyser." Both have the same meaning, but the former seems to me to be more euphonious and to correspond better to the Greek form of the word, although it may have the suggestion of human personality.

If now we turn back to the examples given at the beginning of this chapter, we see at once that they belong to those called catalytic, in that the agent concerned, acid or tissue extract, does not itself form a part of the final chemical system in equilibrium. Again, considering the first of these, the ester system, we note that the catalyst does not actually set into action a new process, but merely hastens one that was already in progress. Ostwald (1902, II., 1., p. 515; 2nd edition) therefore defines a catalyst as a substance that increases the rate at which equilibrium is reached, but at the same time he points out that the reaction, without the catalyst, may be so slow that it appears not to take place at all.

A simple experiment will assist us in understanding the essential properties of a catalyst and avoid confusion with some other processes, which have a superficial resemblance to those of catalysis.

Take a piece of carefully-cleaned, polished plate glass about a metre long and some 20 c. broad. Rest one end on the table and raise the other end on an adjustable support. Now take a brass weight of about one kilogram, polish the bottom and place it on the top of the glass plate, which forms an inclined plane. By delicate adjustment of the angle of the plane, it will be found possible to find such a position that the weight slides down very slowly. This is the most difficult part of the experiment, since a speck of gritty dust will stop the descent, so that it is well to polish the surface with a little talc and a chamois leather immediately before the weight is placed thereon. This part of the experiment represents a reaction taking place of itself very slowly. Apply, next, a little oil to the bottom of the weight and again place it at the top of the plane. It will slide down with great rapidity. The oil represents the catalyst.

There are several instructive points about this scheme. Notice first that the energy available in the "reaction" is simply that due to the fall of the weight from the vertical height of the top of the plane to that of the lower end, and that this is unaffected by the addition of the catalyst, which therefore takes no part in the final state. A point of importance in relation to the catalytic reactions in the living organism is, however, that the form of the energy may be different in the two cases. Without the oil, the weight arrives at the bottom with very little kinetic energy, most of its potential energy having been lost as heat, due to friction along the glass. With oil, very little energy is lost as heat and the

weight arrives at the bottom with considerable kinetic energy. This teaches us that the actual products of a catalysed reaction are not necessarily identical with those obtained in the absence of a catalyst.

The next point is that, within limits, we can vary the rate of fall by the application of much or little oil. Although the catalyst does not affect the position of the equilibrium point, the rate at which this is reached is directly proportional to the amount of the catalyst present. Moreover, comparing the relative efficiency of different amounts of oil, we note that small amounts produce at first a much greater effect than the same amounts added after there is already a considerable amount present. This is characteristic of adsorption and applies to enzymes, the catalysts of living organisms, particularly.

We may next note the difference between what is sometimes called "trigger action" and catalysis. Suppose that the plane is, for convenience, raised to a rather steeper position than before, and that the weight is prevented from sliding down by the support of a catch of some kind. When the catch is removed, the weight falls, but the amount of work done in moving the catch has no effect whatever on the subsequent process; whether the trigger moves very stiffly or easily, the weight descends at the same rate. The true catalyst, oil, exerts its action throughout the whole of the descent, whereas the action of the trigger is completed before the fall begins. Supersaturated solutions are cases of "trigger action." They remain indefinitely as such until infected with a crystal, and then the rate of crystallisation is independent of the amount of crystals added. The same fact is exhibited in the case of supercooled acetic acid, as shown by B. Moore (1893) in his experiments, in which a long tube was used.

One more fact, the meaning of which will be appreciated later, is that in our model the oil partially disappears by sticking to the glass, so that the whole of it is not present on the weight at the bottom. In a certain sense we may say that it has "combined" with some other constituent of the system. In some catalytic reactions we meet with phenomena of this nature; for example, in the chamber process of sulphuric acid manufacture, the nitric acid, which acts as a catalyst, slowly disappears, being used up in subsidiary reactions.

It is held by some that a catalyst may actually start a reaction which was not in progress on account of chemical "friction." Our model, again, shows this phenomenon. The friction between the weight and the glass may be so great that no movement appears to take place until oil is applied. The question is rather of theoretical interest and may almost be said to be one of words. The use of the word "friction" implies the possibility of movement, and it may be said that the weight really does move, but is arrested again. There are also all degrees of friction, with corresponding rates of movement, and the rate of a reaction may be so slow that it is, in practice, impossible to say whether it is actually proceeding or not.

The remark may be made here, that the number of reactions known to be capable of catalytic acceleration is very large and increasing every day.

Discussion of the *mechanism* of catalysis will best be deferred. It is probably of a different nature in different cases.

Before passing on to the subject of enzymes proper, a few words are necessary with respect to *reversible reactions* in relation to catalysis.

In the example chosen to begin with, namely that of the action of acid on the ester system, we saw that the position of equilibrium is unaltered by the presence of the catalyst. Now this position of equilibrium is due to the simultaneous existence of the two opposite reactions of hydrolysis and synthesis, which are proceeding at an equal rate at this moment.

In order to see how the actual position of this equilibrium depends on the relative rate of two opposite reactions, we may take a rough illustration, which must not be followed in too great detail. Suppose that two people start to walk towards one another from two distant places. Where they meet will clearly depend on the relative rates at which they walk. Supposing that their rates are the same, they will meet half way between the places from which they start. Imagine that one of them is excited, "catalysed," so that, instead of walking, he runs. He will meet the other man before he has taken many steps from home. It is also obvious that, if one ran, the only way by which the two could meet at the same place ("equilibrium position") is that the other man runs also, and at the same rate. He must be equally "catalysed" in fact.

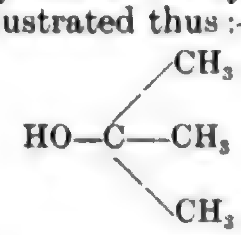
We see, therefore, that the catalyst, acid, must act on *both* the hydrolytic and synthetic components of the reactions. That this is actually the case has been

shown by experiment; indeed, acid is commonly used both for hydrolysis and for synthesis of esters and other compounds. Another point, which is illustrated in the example chosen, is that it is not necessary that the two men should be equally excited by the same cause. If the one ran faster than the other, they would meet at a different equilibrium point. What is important to notice is that if they meet *anywhere* except at either extreme end, they must *both* have been accelerated. The conclusion to be drawn is that, if any slowly progressing reversible reaction is acted on by a catalyst, and the equilibrium position found to be anywhere except at nearly complete hydrolysis or synthesis, *both* of these processes must have been accelerated by the catalyst. In theory, therefore, every catalyst is capable of both hydrolytic and synthetic action and, instead of requiring special proof that an enzyme is a synthetic agent, proof must be demanded of any contrary statement. In the case of the ester acted on by lipase, if the hydrolytic reaction alone were accelerated, the synthetic reaction, going on at its own extremely slow pace, would not have proceeded to any perceptible extent before the hydrolytic one was complete. The equilibrium point would inevitably be close to that of complete hydrolysis, instead of being somewhere near one-third of the distance from the synthetic end.

This point of view is particularly insisted on by van't Hoff (1901, p. 211), and it is interesting to note that, at the time of his death, he was engaged in researches on enzymes with regard to their synthetic action (Cohen, 1912, pp. 575-576). He had already made important advances in the elucidation of glucoside formation (1910), but, unfortunately, never reached the third part of his programme, the processes in the living organism. There is a pathetic interest attached to these latest researches of the great investigator, in that, as Ostwald says (1912, 1, p. 515), they were paid for with parts of his life itself. A portrait of van't Hoff in 1899 has been given in Fig. 24.

There are, in practice, many cases where the equilibrium position is so near complete change in one direction that it is held by some workers in this field to show absence of any reverse reaction whatever. Such a case is the action of emulsin on salicin; even under such conditions of concentration that synthetic action would be most favoured, there appears, to a hasty observer, to be complete hydrolysis. Closer investigation shows, however, that the reaction is not quite complete. Both Visser (1905) and Bourquelot and Bridel (1913) found this incompleteness to be the fact and what is of importance is that both sets of independent experiments gave the same equilibrium point.

This fact obviously means that, even when accelerated by a catalyst, the synthetic reaction is very slow, compared with the hydrolytic one, when emulsin acts on salicin, or its components. The explanation was given by van't Hoff (1910), who pointed out that salicin is the glucoside of a tertiary alcohol, that is, of an alcohol which contains a carbon atom united directly to three other carbon atoms and with its third valency united to hydroxyl (see Bunge-Plimmer, 1907, pp. 71-73). The group may be illustrated thus :—



which is tertiary butyl alcohol.

Now, according to the work of Menschutkin (1879) on esterification, tertiary alcohols are very difficult to esterify, and van't Hoff shows that the same statement applies to the formation of glucosides. A primary alcohol, which contains the group CH_2OH, on the contrary, is easily esterified or made into a glucoside. The alcohols of our first experiments, iso-amyl and ethyl alcohols and glycerol, are all primary, so that the synthetic action is easy and the equilibrium position is a considerable distance from both ends. The synthetic reaction, in those cases where the equilibrium point is close to that of complete hydrolysis, is one that is of inherent chemical difficulty, and the facts given above with respect to the equilibrium position are thus to be accounted for. We shall see later, however, that even a small amount of synthesis is of considerable importance under such conditions that the product is removed as fast as it is formed.

A point of significance, in view of the mode of action of catalysts, is that they exercise a powerful action even when present in very *minute amounts*. This is not surprising when we remember that they usually reappear at the end of the reaction in an unaltered state, and are therefore ready for further work. We have already seen that the position of equilibrium under a particular kind of catalyst is not affected by the amount of this catalyst present. What is observed is that the time taken to attain equilibrium is shorter with the larger concentration of catalyst. This fact is a useful criterion in deciding whether, in a particular case, we have to deal with a catalytic process or with one in which the constituents enter into combination in molecular proportions. In the latter case, of course, the *amount* of product will depend on the *amount* added of the reagent whose nature we desire to test. Although it is not a matter for surprise that the final effect of a catalyst should be independent of its amount, it is striking to note how very minute are the quantities which are able to produce considerable results.

For instance, Brode (1901, p. 289) found that the reaction between hydrogen peroxide and hydriodic acid was appreciably accelerated by the presence of 1 gram-molecule of molybdic acid in 31,000,000 litres. Again, a preparation of invertase, which probably consisted only to a small percentage of the active catalytic agent, was found by O'Sullivan and Tompson (1890) to hydrolyse 200,000 times its weight of cane-sugar. These facts will serve to impress upon the reader the point that the amount of *chemical energy* which a catalyst is capable of supplying to a reaction is negligible, and the assumption cannot be used to explain any of the phenomena. This will be referred to again.

Heterogeneous Systems.—We shall find presently that the particular catalysts of especial interest to us are in the colloidal state. We have, indeed, seen already, in our first typical examples, that lipase and emulsin act in liquids in which they are completely insoluble. It is therefore necessary to consider briefly the mechanism of reactions in systems of more than one phase. The theory of these reactions is due chiefly to Nernst (1904). They may be said to take place in three stages. Suppose that the catalyst is present in the form of solid particles, and that the other components, which are to be brought into reaction, are in true solution. In order that they shall be influenced by the catalyst, it is obviously necessary that they shall diffuse to it, since it is not uniformly distributed throughout the system. The rate of *diffusion* is the *first* factor. If these solutes lower surface energy, as practically all solutes do, they will next be concentrated by adsorption at the interface between the catalyst and the solution. *Adsorption* is the *second* stage. The *third* stage is the *chemical* reaction proper. It is clear that the increased concentration on the surface will, in itself, hasten the reaction by mass action, and this was, in fact, the explanation suggested by Faraday (1839, 1, p. 184) for the effect of platinum in causing combination of oxygen and hydrogen. Whether all cases of heterogeneous catalysis can be explained on these lines is doubtful. In certain cases of catalysis in homogeneous systems, as we shall see later, there is an intermediate compound formed between the catalyst and the reagents; but it is certain that this does not apply to all cases; indeed, it appears to be exceptional.

Faraday's views on the possibility of the close approximation of oxygen and hydrogen on the surface of platinum being sufficient to cause their molecules to enter into combination led to a long discussion with De la Rive, who held that there is an intermediate formation of some oxide of platinum. With our knowledge of Faraday's wonderful insight into the mechanism of natural phenomena, we may well be inclined to think that he was most likely on the right side in this case. Kohlrausch remarked, "Er riecht die Wahrheit," "he smells the truth" (see Tyndall's "Faraday as a Discoverer," 1870, p. 55).

In the discussion of this question, it is to be remembered that there is reason to believe that it is during the actual process of condensation itself that the molecular stresses result in unusual chemical activity (see Hardy's note to the paper by Drury, 1914, p. 175).

In all heterogeneous reactions, the rate of the reaction, as measured, is naturally that of the slowest member of the series. Adsorption is a rapid process, when the substances are in contact, so that the rate of the reaction will be either that of diffusion or of the chemical component of the reaction. When the catalyst is in colloidal solution, the length of the way to be passed over by diffusion is very short, since the active substance is almost uniformly distributed; so that the chemical reaction itself, unless of great rapidity, controls the rate of the whole

process. When a metal in mass is immersed in acid, the diffusion process is slower than the chemical reaction.

When one substance is adsorbed on the surface of another, it does not follow of necessity that any chemical reaction will occur. Aniline on the surface of mercury in Lewis' experiments (1910, 3) may be given in illustration. When chemical reaction does occur, the rate at which it proceeds is obviously controlled by the amount adsorbed at any given moment, so that an exponential relation between the concentration and the velocity is to be expected.

The mode of action of catalysts, with especial reference to enzymes, will be discussed later.

ENZYMES AS CATALYSTS

We have seen that certain substances, extracted from animals and plants, act in a catalytic way similar to that in which an inorganic compound, such as acid, does. These substances are known as "enzymes" or "ferments."

In the discussion of their properties, certain names will have to be used, so that the terminology of the subject must first be referred to. The choice of correct words is really more than a mere matter of convenience. If the word used has a meaning, is connotative, it should tell us something about the thing named, although it frequently happens that the original meaning becomes changed as knowledge increases. As often pointed out, the progress of a science depends much on the language used in the description of its phenomena. It is a mistake, however, to be hasty in inventing new names; more care must be exercised in attaining certainty that the new name is required to describe phenomena of a new kind, inadequately provided for by names already in use. Numerous names, at one time thought necessary, have disappeared.

As various substances were extracted from organisms, and the similarity of the action of these substances to that of alcoholic fermentation became obvious, it was natural to call them "ferments." And when Cagniard de Latour (1838) showed that alcoholic fermentation was due to a living organism, substances such as the "diastase," precipitated by Payen and Persoz (1833) from extracts of malt, were distinguished as "soluble," "unorganised," or "unformed" ferments from "living," "organised," or "formed" ferments. In process of time some confusion was caused by this double use of "ferment," so that Kühne (1878, p. 293) thought it well to introduce a new name for the soluble, or unorganised ferments. The passage is sufficiently interesting to be translated here:—

"The latter designations (formed and unformed ferments) have not gained general acceptance, since on the one hand it was objected that chemical bodies, like ptyalin, pepsin, etc., could not be called ferments, since the name was already given to yeast-cells and other *organisms* (Brücke); while, on the other hand, it was said that yeast-cells could not be called *ferment*, because then all organisms, including man, would have to be so designated (Hoppe-Seyler). Without stopping to inquire further why the name excited so much opposition, I have taken the opportunity to suggest a new one, and I give the name *enzymes* to some of the better-known substances, called by many 'unformed ferments.' This name is not intended to imply any particular hypothesis, it merely states that ἐν ζύμῃ (in yeast) something occurs that exerts this or that activity, which is supposed to belong to the class called fermentative. The name is not, however, intended to be limited to the invertin of yeast, but it *is* intended to imply that more complex organisms, from which the enzymes, pepsin, trypsin, etc., can be obtained, are not so fundamentally different from the unicellular organisms as some people would have us believe."

On account of the important work done by Kühne in the elucidation of the action of enzymes, I introduce his portrait in Fig. 83. The name "enzyme" has come into general use, although "ferment" is still to be met with as synonymous with it; while the application of this name "ferment" to living organisms has dropped out of use.

Enzymes may be shortly defined as the catalysts produced by living organisms. If we grant that the substances known by the name are a special kind of catalysts, which we have still to show, it is clear that the introduction of a name for them is merely a matter of convenience. At the same time, the majority of them have

certain incidental properties which distinguish them from the majority of inorganic catalysts, but this is all that we are justified in asserting.

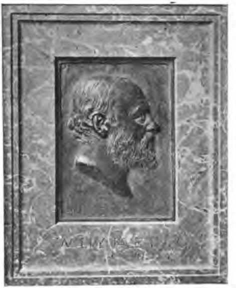
It is interesting to note that Von Wittich (1872) had already come to the conclusion that pepsin merely accelerates the action of hydrochloric acid on fibrin, but it is not quite clear whether he intended to make the statement that enzyme actions in general are catalytic actions, although it appears to be so.

A name is often wanted for the substance on which an enzyme acts. No satisfactory one has been suggested. "Hydrolyte" excludes all processes except hydrolysis, "zymolyte" applies only to enzymes and excludes other catalysts, a suggestion of difference which is to be deprecated. "Substrate" is in very general use; it is rather an awkward word in English, but will be used in the following pages.

To distinguish the different enzymes, Duclaux suggested adding the termination "ase" to the name of the substrate, thus "lactase" is the enzyme which acts on lactose. Certain old names, such as "pepsin" and "trypsin," are, however, still in use.

The termination—"lytic"—has been used for a class of enzymes acting on a group of substances; a proteolytic enzyme is one that acts on the proteins in general, and includes pepsin and trypsin. Armstrong (1890) has justly pointed out that "proteolytic," in analogy with "electrolytic," should mean decomposition by means of protein, not decomposition of protein itself. To avoid this misuse, the termination "-clastic" was suggested, and I will attempt to make consistent use of it.

A recent unfortunate introduction of "ese" as a termination for an enzyme which acts synthetically only has been shown to be unwarranted (Bayliss, 1913, 1) and need not concern us further.



Ihr ergebener

W Kühne

FIG. 83. W. KÜHNE.
(Photograph of the Tablet in the Institute of Physiology, Heidelberg.)

We have seen that Berzelius himself placed the substances which we now call enzymes in the class of catalysts. Apart from theoretical interest, it is of some practical importance to know what kind of properties may be expected to be shown by a substance supposed to be an enzyme.

The only really *essential property of a catalyst* is that it changes the rate of a reaction, including the starting of one which does not appear to proceed of itself, and without entering as a constituent into the final chemical equilibrium. There are certain other properties usually present, but not essential, although they are sometimes of assistance in deciding the nature of particular cases. We will now proceed to inquire how far enzymes satisfy the above conditions.

It may be useful, first of all, to mention a few *typical enzymes*, remembering

that there are new ones continually being discovered. How far many of these new ones are really such, and not new capabilities of old enzymes, may sometimes be a matter of doubt. In the following list, the name of the appropriate substrate is placed in brackets after that of the enzyme: amylase (starch), maltase (maltose and α-glucosides), emulsin (β-glucosides), pepsin (proteins in acid medium), trypsin (proteins in alkaline medium), urease (urea), arginase (arginine), lipase (esters), peroxidase (organic peroxides, including that of hydrogen), and so on. We may also divide enzymes into classes according to the nature of the chemical change accelerated. The majority add or remove the elements of water, and may be called *hydrolysing* from the one aspect of their activity. All of those mentioned above, with the exception of peroxidase, belong to this large class. Those that cause activation of oxygen or of hydrogen, bringing about oxidations and reductions, will be dealt with in Chapter XX. There is another class which appear simply to break up a complex molecule, although it is probable that this is done by a series of changes, involving oxidation, reduction, and hydrolysis, as in the case of the zymase system of yeast, converting glucose into alcohol and carbon dioxide—

$$C_6H_{12}O_6 = 2C_2H_6O + 2CO_2.$$

Whether an enzyme merely accelerates a spontaneous reaction it is impossible to state as a general rule. But there are certainly some reactions which proceed slowly by themselves and are accelerated by enzymes: the esters in water may be mentioned. In other cases, the change, rapid under the action of an enzyme, is, at ordinary temperatures, too slow to be detected, although it can be made to proceed at a measurable rate by raising the temperature; the hydrolysis of cane-sugar and of salicin by water are cases in point. When a reaction can be made obvious by heat, it is justifiable to conclude that it is not entirely absent at ordinary temperatures.

In such cases of solutions in water, the question arises as to whether the spontaneous change might be due to the catalytic action of the small quantity of hydrogen and hydroxyl ions always present. Consideration will show, however, that, even when a reaction is proceeding under the influence of one catalyst, if it is further accelerated by another substance, this second is no less an additional catalyst; except in those cases where the second acts by increasing the activity of the first, and is inactive alone.

A more important point is the question of the relation of the enzyme to the *final products*. In some cases the enzyme has been recovered at the end of the reaction unchanged, as the acid in ester reactions. In other cases it disappears, partially or entirely. This disappearance, however, is found to be due to the instability of the enzyme itself. That it does not form a component of the final equilibrium is shown by the numerous experiments in which it has been found that the total amount of change is independent of the amount of enzyme added, which would be impossible in the other case. A series of curves illustrating this fact will be found in my monograph (1919, 1), which shows also how the *rate* of the change depends on the amount of the catalyst. Another case, using the synthetic aspect of the action of emulsin, is given in Fig. 84 below.

If the enzyme formed a component of the final equilibrium, the position of this equilibrium would be altered by mass action if more enzyme were added after its attainment. This, in point of fact, does not happen (Bayliss, 1913, p. 246).

Certain views as to the attainment of what has been called a "false equilibrium," in which the final result appears to be in proportion to the concentration of the enzyme, will be found discussed in a paper by myself (1913). It will suffice to say here that careful examination of the experimental facts shows that they do not compel us to make an assumption of this kind, and are, for the most part, to be accounted for by destruction of the enzyme before it has had time to carry the reaction as far as the equilibrium position. Naturally, the more enzyme is present at first, the faster the reaction proceeds; and, moreover, it will be longer before the whole of the enzyme has disappeared.

The fact that the position of equilibrium is found to be the same whether we start from the system consisting only of substrate or only of products, is again of considerable importance as regards the proof that we are dealing

with a true equilibrium in a reversible reaction (see Figs. 80 and 81, page 300 above).

We see then that enzymes are, beyond doubt, typical catalysts in the comparatively simple cases hitherto considered. Since, however, many of the reactions taking place in the living organism under the influence of enzymes are of a complex chemical nature, not as yet completely understood, it is not to be wondered at that we meet with phenomena which seem, at first sight, to be difficult to reconcile with the hypothesis of catalysis in reversible systems. We shall presently meet with further evidence that, in cases of enzyme action where we have all the factors under control, the reactions obey all the laws they would be expected to do on the hypothesis mentioned. It appears to me that we are hereby justified in holding that the more complex cases, such, for example, as those where proteins are concerned, will be found to require no assumptions contrary to the laws obeyed in the simpler cases. In these heterogeneous, colloidal

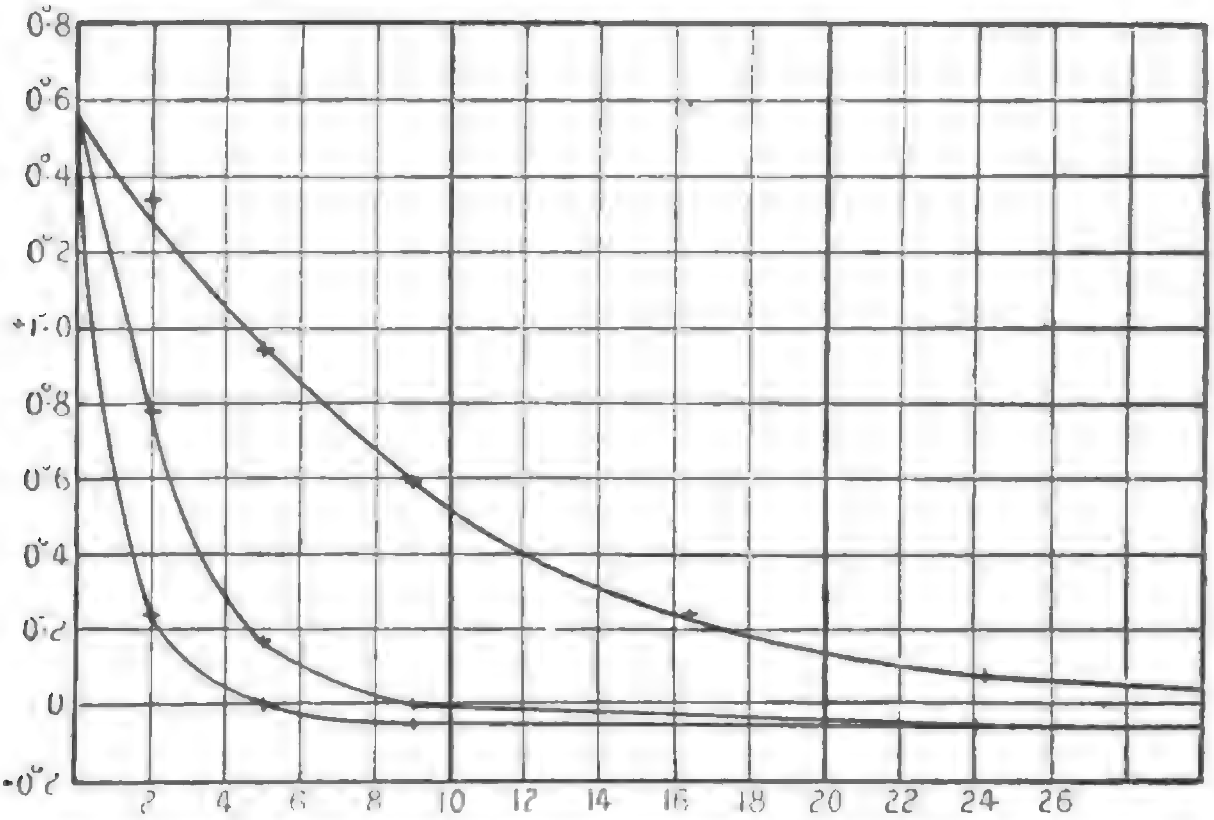


Fig. 84. Rate of synthesis of glycerol-glucoside with different concentrations of emulsin.—The ratios are as 1 : 4 : 12, the uppermost curve having the lowest concentration. All arrive finally at the same position of equilibrium.

(Bayliss, 1913, p. 246.)

systems, the facts brought forward in the preceding chapters are sufficient to indicate what innumerable possibilities of modification are present, in the way of surface action, electric charge, and so on. It is the work of the future to investigate the intervention of these factors in the course of the chemical reactions brought about by the various individual enzymes.

Certain incidental properties common to inorganic catalysts and enzymes serve to strengthen our position. The fact that very minute quantities are active has been mentioned already: how minute the really active substance is in the case of enzymes we do not know, owing to the difficulty of preparing them in a chemically pure state from the complex mixtures in which they are found.

In the definition of enzymes, we sometimes find the qualifications introduced that they are *colloidal*, specific catalysts destroyed by heat [E. F. Armstrong (1913)]. It is true that the substances which we separate as active enzymes are practically all in the colloidal state, but we are not certain that this state is necessary to their activity in all cases. As to *specific* nature, the circumstance that a particular catalyst acts on a limited class of substrates is not peculiar to those produced by living organisms; some inorganic catalysts are very specific as, for example, tungstic acid is a powerful catalyst for the oxidation of hydriodic acid by hydrogen peroxide, but not for its oxidation by persulphates or by bromic acid. On the other hand, some enzymes are not particularly specific, emulsin acts on the whole series of

β-glucosides, which are almost innumerable in number. Some investigators seem to be prepared to postulate a separate enzyme for each glucoside. This question requires more detailed discussion in a later page.

As to the action of *heat*, the sensibility of enzymes varies considerably, according to the conditions present. As a rule, they are coagulated or precipitated by heat, but in some cases the enzymes seem to be merely carried down by adsorption or changed in their physical state, reversibly. In practice, however, this property is frequently useful in deciding the nature of a particular agent. If the action is stopped by moderate heat, say up to that of boiling water, it is almost certainly due to an enzyme or to the action of living protoplasm, using this latter name for the present as a cloak for ignorance. To distinguish an enzyme action from it, use is made of *antiseptics*, which have a more powerful action on what we call "vital activity" than on that of enzymes. But, again, the distinction is one of degree only, some enzymes are very sensitive to antiseptics, others not; the difference probably depends on complexity of structure. Invertase is comparatively insensitive, zymase is very sensitive. Meyerhof (1913 and 1914) finds the inhibiting effect of indifferent narcotics on enzymes to be completely reversible, and interprets it as being due to the driving off of adsorbed substrate by the more strongly adsorbed narcotic. We shall see later that preliminary adsorption is a phase in the action of enzymes.

We may take it, then, that enzymes are a special class of catalysts; this being so, their function is to *alter the rate* of reactions. The factors involved in the velocity with which a reaction takes place have been incidentally dealt with to some extent, but it may be well to spend a little more time on the question, as it affects catalytic action especially.

VELOCITY OF REACTIONS

When one large molecule is undergoing the process of being divided up into smaller ones, spontaneously, or under the action of a catalyst, there is no difficulty in seeing that the number of molecules split in a given time is proportional to those present.

This is the law of mass action in its simplest form. The history of this law, which is the foundation both of chemical statics and dynamics, will be referred to below, under the head of equilibrium.

If C is the concentration at the time t, then $\frac{dC}{dt}$ is the velocity of change during so short a time that the rate does not alter, and, according to the law of mass action,

$$\frac{dC}{dt} = kC,$$

where k is a constant, varying with each individual case and known as the velocity constant.

In order to use this equation for any practical purpose, it must, of course, be integrated, so that the change during a measurable time can be investigated. The reader will note that we have another case of the "compound interest law," and the simplest form of the integral is

$$k = \frac{1}{t_2 - t_1} \log_e \frac{C_1}{C_2},$$

where C_1 and C_2 are the concentrations of the molecule undergoing change, t_1 and t_2 are the times after the commencement of the reaction at which the concentrations are found to be C_1 and C_2. This is known as a *unimolecular reaction*.

It is obvious that, in practice, any quality of the substance concerned which is a mathematical function of its concentration, such as electrical conductivity or optical rotation, can be used to represent C, provided that it is known what function of the concentration this quality is.

Let us take a step further and suppose that the reaction is one in which two

molecules combine together to form one or more different ones. This is a *bimolecular* reaction, since the change of concentration of two molecules must be taken into account. What is the law here? Take for a moment the kinetic point of view and suppose that we increase the concentration of the one reacting component, A, and leave the other, B, intact; the rate is clearly proportional to (A), using brackets to express concentration, since the number of times that a molecule of B meets with one of A is proportional to the number of A to be met with in a given space. Suppose that we increase (B), leaving (A) alone, then the number of times collision takes place is proportional to (B); therefore, if both are changed, the velocity of the reaction is proportional to the product of the concentrations of the two molecules, or

$$\frac{dx}{dt} = k(\mathrm{A})(\mathrm{B}).$$

In practice, most cases of bimolecular reactions can be simplified for integration, since the concentration of A and B can be made to change equally. In the saponification of esters, say ethyl acetate, by sodium hydroxide, the equation is:—

$$\frac{dx}{dt} = k(a-x)(b-x),$$

where a and b are the initial concentrations of ester and alkali, and x is the amount of sodium acetate produced in the time t. If equivalent quantities of ester and alkali are taken, this equation becomes

$$\frac{dx}{dt} = k(a-x)^2,$$

since $a = b$. The integral of this equation is

$$k = \frac{1}{t} \cdot \frac{x}{a(a-x)}.$$

Hydrolysis of Cane-Sugar by Acids.—This process can clearly be expressed as a unimolecular reaction, since it is completely accounted for, as regards its rate, as the change of concentration of the one kind of molecule, cane-sugar. Applying the formula to it, it is found that the constant k is the same at all stages of the reaction. Indeed, as mentioned before, the determination of this velocity constant has been used as a method of determining the hydrogen ion concentration of various acid solutions.

Application to Enzymes.—Suppose that cane-sugar is hydrolysed by the enzyme invertase, instead of by acid. What sort of values of k do we obtain? The value of making measurements of this kind in the case of enzymes is that we thereby obtain indications as to what to look for as causes of any divergence found, and also, by the regularity of the time course of the divergence, we are able to estimate the accuracy of the method of experiment adopted.

In all cases where we are investigating the action of an enzyme on a single substrate, as in the majority of hydrolytic reactions, we might expect to find that the unimolecular equation is followed. In point of fact, in the case of invertase, if we calculate the velocity constant by the unimolecular formula, we find that it steadily rises as the reaction proceeds; in a particular case, from 0·00058 to 0·00097 (Victor Henri, 1903, p. 55). Taking other enzymic actions, we find, on the contrary, almost invariably, a decrease in the value of k. E. F. Armstrong (1904, 1, p. 506) found it to fall from 0·0640 to 0·0129 in the case of lactase.

We may now proceed to find what possible factors might have the effect of diminishing the rate more than it should be, by mass action, on account of the mere diminution in the number of molecules of the substrate, leaving, for the present, the exceptional case of invertase.

There are two things to be kept in mind with regard to this question. The first is that there is every reason to suppose that all the reactions with which we are dealing are reversible, and that there are two opposite reactions proceeding simultaneously, so that the net result observed in any experiment is the difference between the rates of these two reactions. Suppose that our reaction is the

hydrolysis of a substrate into simpler molecules. As the equilibrium position is approached, the opposing synthetic reaction becomes more and more marked by mass action of the increasing products of hydrolysis, and, at the equilibrium position, becomes equal in rate to the hydrolytic one. It is unnecessary to remind the reader that what is spoken of here is the actual rate, that is, the total amount of change in a given time, not the velocity constant, which is, of course, independent of the active masses.

Under the conditions in which most enzyme experiments are made *in vitro*, the equilibrium position is so near that of complete hydrolysis, owing to the excess of water present, that the synthetic reaction is too small to exercise any very perceptible influence, so that other causes for the slowing of hydrolysis must be sought for. When the conditions are such that the synthetic reaction is considerable, as in the cases of lipase and emulsin quoted at the beginning of this chapter, the reversibility of the reaction plays an important part. Fig. 80 (page 300) shows how the hydrolytic reaction in the case of lipase is favoured by the presence of water.

The second thing to be considered is that the rate of a catalysed reaction is dependent on the concentration of the catalyst. If, then, anything happens during the course of the reaction which diminishes the amount of enzyme present, either actually, by destruction, or effectively, by paralysing its activity, the result will be a progressive diminution in the rate of the reaction. We note that this disappearance of enzyme may be irreversible, when there is actual destruction, or reversible, when it is merely removed from the sphere of action, either by temporary paralysis, caused by the products of the reaction, or by adsorption on the surface of some substance present in the system, as is the case with many of the so-called "*anti-enzymes*."

It is well to mention here that the particular cases which are used in the following pages for illustrative purposes are not to be taken by the reader as the only ones of the kind known. Numerous others will be found in my monograph (1919, 1).

We will take first the question of the actual *destruction of enzyme*. A solution of any enzyme is found to lose its activity if kept. This is, in great part, due to the complex colloidal state of these substances. The rate of this loss of activity varies very much according to the individual case, and is accelerated by rise of temperature. The time course of the process was investigated by Tammann (1892, 2) in the case of emulsin. In the presence of substrate the rate of destruction is much decreased (Bayliss and Starling, 1903; Vernon, 1904), although not entirely prevented. This protective action of substrate has been ascribed to chemical combination with the enzyme. Without denying that this may sometimes occur, I have found that mere adsorption (Bayliss, 1911, 1) by charcoal is protective in the case of trypsin, and there is no proof that this may not be the general explanation.

With very low concentration of enzyme, even in the presence of substrate, activity has been found to disappear before completion of the reaction (Tammann, 1892, 2, and Bayliss, 1913, 1, p. 248); this fact has led to certain erroneous statements with regard to "*false equilibrium*." On the other hand, experiments made for the purpose (Bayliss, 1904) have shown that, in the case of trypsin, there is no detectable loss during a few hours, although the velocity constant has diminished considerably. The spontaneous destruction of enzyme is, therefore, not the sole cause of the decrease in activity.

Reversible Inactivation.—It is found by experiment that the addition of certain substances, amongst which are to be found the products of the reaction itself in a large number of enzymic reactions, has a great effect on the activity of enzymes. This action is brought about in several different ways:—(1) Enzymes are colloids, and therefore liable to aggregation or precipitation by a variety of agents. Emulsin is precipitated by benzaldehyde, so that, even in the small quantities produced in the course of the hydrolysis of amygdalin, it is probable that a certain degree of aggregation and diminution of active surface is produced. We have seen (page 301) that enzymes act by their surfaces, and further evidence will be given presently. (2) Most enzymes are extraordinarily sensitive to changes of hydrogen ion concentration. This is a common property of the colloidal state and points

to the intervention of electrical charge. The slight diminution in hydrogen ion concentration caused by the addition of blood serum is sufficient to bring about great retardation in the action of emulsin (Bayliss, 1912, 2). Trypsin is inactive except in slightly alkaline reaction, and there is a particular narrow concentration of hydrogen ion in which it, as indeed enzymes in general, are most active. Now it has been shown by Brailsford Robertson and Schmidt (1908-9) that, during the course of a digestion by trypsin, there is a progressive increase of hydrogen ion concentration, or diminution of hydroxyl ion, due to the production of amino-acids, which, combining with the free alkali originally added, diminish the alkalinity, and thus retard the action of the enzyme more and more as the reaction goes on. It is found, indeed, that the addition of amino-acids has a powerful effect in slowing trypsin digestion.

An observation that has probably been made by many workers with trypsin is that, supposing that one starts a digestion of caseinogen with trypsin, adding only the optimal amount of alkali, after a week or two in the incubator one finds that the digest is distinctly acid to litmus, and the rate of action can then be made to increase by addition of more alkali.

A reaction, then, can be caused to proceed more rapidly by removal of the products, as was pointed out by Kronecker (1874), and this result is due, not only to reversibility, but also to the fact that the products are more or less toxic to the enzyme itself.

As would be expected, the sensibility of enzymes to various substances is much greater than that met with in ordinary chemical reactions. It is held by some investigators that there is a special "affinity" of each enzyme for certain products of its action, in that the rate of change is affected more by these than by other related substances. Thus, E. F. Armstrong (1904, 2) found that fructose retards the action of invertase more than the corresponding concentration of glucose does, and the statement is made that invertase is "controlled" by fructose. The fact, however, that fructose also "controls" the action of maltase more than glucose does (Philoche, 1908, p. 243), although it is not a constituent of the system concerned, suggests that the relationship is not one of a chemical nature between the constitution of the enzyme and of its substrate. The excessive sensibility of enzymes to acidity and to some inorganic salts warns us that great caution must be exercised in the interpretation of results of this kind. Bourquelot (1913, 2, p. 3) finds that the hydrolysis of arbutin is retarded by hydroquinone, but not that of salicin. Hence the action is not on the enzyme itself, and increase of the reverse reaction is suggested.

The *acceleration of rate*, in the course of the action of invertase, is probably due to the production of some substance in small amount which increases the activity of the enzyme. Acid does this, and it has been stated that an acid is produced in small quantities during the action of invertase, and it may perhaps be lævulinic acid. So far as I am aware, no measurements of the hydrogen ion concentration during the reaction have been made.

There is a certain similarity between this production by an enzyme of substances which affect its own activity and the process called by Ostwald "*Autocatalysis*" (1902, II. (2), pp. 263-266). In the first illustration at the beginning of the present chapter we saw that the spontaneous hydrolysis of methyl acetate in water is greatly accelerated by the addition of acid. Now in the hydrolysis itself, free acetic acid is formed, which must act as a catalyst, although not a powerful one. Moreover, it increases in amount by its own activity, so that, if we determine the velocity constant at different times, we find that it increases at a greater and greater rate.

In an experiment of this kind which I performed, at the beginning of the reaction the velocity constant was 49×10^{-7}, in nineteen days it had risen to 593×10^{-7}, and in forty-two days to $1,498 \times 10^{-7}$. In half-normal hydrochloric acid, it was initially 1,600 times that in water and equilibrium was reached in about six hours, so that, if there were no autocatalysis in water the attainment of equilibrium would have taken $6 \times 1,600$ hours, or 400 days. This fact may assist the reader to realise how slow the spontaneous reaction is, and how impossible it is for the equilibrium position to be unaltered by acid unless the catalyst accelerated both the hydrolytic and synthetic reactions.

Perhaps a few more details will be useful with regard to the phenomenon of autocatalysis. When the curve of a reaction of this kind is plotted with time as abscissæ and actual rate of change as ordinates, it is found to have an S shape. The rate is slow at first, becomes quicker and then slows again. This course is typical of autocatalysis and is, obviously, due to the deficiency

of catalyst at the beginning and deficiency of substrate at the end; the latter fact causes diminution in rate by mass action. The rate of the reaction, as measured by the amount of ester hydrolysed in unit time, must not be confused with the *velocity constant*, which increases steadily throughout.

The difference between true autocatalysis and the effect on enzymes described above is that, in the former process, the actual quantity of the catalyst is altered, positively or negatively, whereas in the latter the enzyme causes the production of substances which act upon itself in a similar positive or negative way, the effect increasing more and more as the reaction progresses, so that the change of concentration of the catalyst is not actual but only effective.

We may now consider the effect of different *concentrations of enzyme* as added intentionally at the beginning of the reaction. A practical point of some importance may appropriately be mentioned here. As Bredig points out (1902, p. 187), in comparing the results of the action of enzymes under different conditions or concentrations, we ought to compare the reactions *at the same stage*, since, in this way only, can we be certain of having the same proportion of substrate and products, and, moreover, if the reaction goes in stages, we should otherwise obtain very false information. What we must compare, then, are the times taken to effect equal changes, not the changes produced in the same time.

In practice this is most conveniently done by taking series of measurements and plotting them as curves. If amounts of change are made ordinates and time abscissæ, a horizontal line drawn to cut all the curves at the stage desired will give the time values required.

What is always found, except when there is very little enzyme in proportion to the substrate, or vice versa, is an obvious disproportion between the amount of enzyme and its effect. This may be seen in the following table taken from an experiment of my own with trypsin and caseinogen (1904). The first column gives the relative amounts of the enzyme added to the same volume of substrate. The second column gives the times taken by each to produce the same amount of change, measured by the electrical conductivity, that is, the increase of the concentration of carboxyl groups (see page 219 above). The third column gives the mean rate in each case, namely, the reciprocal of the time taken (multiplied by 1,000 to avoid long fractions). The fourth column gives a measure of the activity of the enzyme as obtained by division of the actual rate by the amount of enzyme present, or, in other words, it represents the activity of equal amounts of enzyme when present in different concentrations, and may be called "specific activity."

Relative Concentration of Enzyme.	Time taken for equal Change in Minutes.	Mean Rate × 1,000.	"Specific Activity"
8	41	24	3
5	48	20·8	4·16
4	55	18·2	4·55
2	81	12·4	6·2
1	144	7	7

It is obvious that the smaller quantities of enzyme are considerably more effective in proportion to their concentrations than the larger ones are. Taking the numerical values of enzyme concentrations 2 and 4, for example, we find that the value of 4, instead of being double that of 2, is less than this. Let us suppose that, instead of being multiplied by 2, it is multiplied by some root of 2, 2^{-x}, and let us see what values are to be given to x to satisfy the various data. As a first approximation, try $x = 2$, then the value of enzyme concentration, 4, should be that of concentration, 2, multiplied by 2^{-2}, that is, $12·4 \times 1·4 = 17·4$, instead of the experimental value of 18·2, a fairly satisfactory agreement. This is in fact the rule known as the *square root law* of Schütz and Borissov, but we see that it is merely an approximation. If we take different stages of the same experiment, we find, in fact, that the value of the exponent increases nearly to 2 towards the middle of the reaction, but may be very nearly unity at the beginning. In this latter case

there is linear proportionality. It is also different where the enzyme concentrations are very far removed from one another, or of high values, being smaller if we compare concentrations of 64, 128, and 256, in arbitrary units, with those of 1, 2, and 4 in the same units. Details of these experiments will be found in a paper by myself (1911, 1, pp. 90-94).

The reader will probably notice at once that these results are precisely what we should expect if the velocity were controlled by adsorption; it was, in fact, this relationship which first led me to suggest the hypothesis of adsorption as applying to the case (1906, p. 224).

It is then impossible to formulate a general law, correlating the concentration of enzyme with its activity, and capable of giving numerical results, except one of considerable complexity. Each case must be investigated for itself until we know more of the changes taking place in the colloidal state of the enzyme during the course of the reaction. This state is, no doubt, the cause of the variations of the adsorption exponent which we have met with; it may have any value between one and two, values above two are rare.

There are three classes of substances which may next be considered, since they affect the rate at which the enzyme acts.

1. *Electrolytes.*—Pepsin and trypsin are inactive except in acid or alkaline solutions respectively. Amylase requires neutral salts, which have also a beneficial effect, as a rule, on enzymes generally (Bierry, 1912).

2. *Co-enzymes.*—Bertrand (1897) found that the oxidase of Japanese lacquer is ineffective without the presence of manganese and called this substance the "co-enzyme" of laccase. These oxidation systems will be considered in Chapter XX.

Magnus (1904) discovered that the lipase of the liver loses its activity when dialysed, but recovers it when bile salts are added. It seems probable that this action is exerted on the enzyme itself, since it applies to the action on soluble esters as well as to that on fats, which might be supposed to be better emulsified by bile salts. These latter, having so great a power of lowering surface tension, are no doubt able to bring about a greater colloidal dispersion of the enzyme, thus increasing its active surface.

Another interesting case of a co-enzyme is that of *alcoholic fermentation.* Yeast juice contains an enzyme, or rather enzyme-system, "zymase," which brings about the formation of alcohol and carbon dioxide from sugar. Harden and Young (1906) showed that such juice, filtered through Martin's gelatine filter, which keeps back the colloids, was separated into two constituents, neither of which was active by itself, but became so on mixing again. Since inorganic phosphates increase the activity of yeast juice, it was thought that they might be the co-enzyme, but experiments showed that these alone were incapable of restoring activity to the colloidal matter left on the filter, and that it was necessary to add boiled yeast juice in addition. Both substances are indeed required.

3. *Anti-enzymes.*—When a foreign protein is injected subcutaneously into an animal, some kind of a neutralising substance is produced. This is known as the "anti-body," while the injected substance producing it is the "anti-gen." There is as yet no satisfactory proof that any substance other than a protein can act as an antigen. The antibodies are of various kinds, sometimes they precipitate the antigen and are known as precipitins, sometimes they act in neutralising its toxic properties in some other way and are called "anti-toxins," sometimes they cause agglutination of bacteria, "agglutinins," and so on.

Now statements have been made that when enzymes are used as antigens, anti-enzymes, true antibodies in the above sense, that of Ehrlich, are formed. It is to be noted that a true anti-enzyme must be specific and act only on the particular enzyme which caused its production, its antigen. It is therefore incorrect to describe any substance which retards the action of an enzyme as an anti-enzyme; otherwise, alkali would have the right to be called "antipepsin."

The results of certain experiments which I had occasion to make with

regard to emulsin (1912, 2), in which rabbits were injected with the enzyme, led me to examine carefully the evidence as to the existence of anti-enzymes. In the experiments referred to, it was found that a "precipitin" was formed for the vegetable protein present as impurity in the emulsin used, but that there was no precipitin for the enzyme itself. The serum, in point of fact, did retard the action of emulsin, but the effect was found to be merely due to diminution of the acidity of the solution; when this was brought back to its initial value by the addition of acid phosphate, the inhibitory effect disappeared. Moreover, making an emulsin solution of the same hydrogen ion concentration as that produced by the addition of "immune-serum" caused the same degree of retardation.

It is to be noted that "anti-emulsin" was the first anti-enzyme supposed to be produced (Hildebrandt); it is generally regarded as a typical one and certainly has more evidence in its favour than any other one. This evidence is discussed in my paper referred to above. When the serum of an animal shows, normally, "anti-enzymic" properties, it is naturally impossible to obtain satisfactory evidence that these can be increased by the injection of the enzyme in question, since the property exhibits large natural variations. In other cases, adsorption of enzymes by colloidal substances is sufficient to account for the "anti" properties; Hedin (1906) showed that the adsorption of trypsin by charcoal is precisely similar to a typical retardation by anti-enzymes.

Thaysen (1915) finds that the so-called "anti-rennin" of serum is to be entirely accounted for by the two influences referred to above, the adsorption of the enzyme on the one hand, and the effect of change in hydrogen ion concentration, as found by myself in the case of emulsin. There is no true antibody formed.

We shall see later that enzymes are not proteins, at least the fact has been definitely established in some cases and in none is there evidence of their being so. This, in itself, is *à priori* reason for doubting the production of true antibodies, until it has been shown that substances other than proteins can give rise to their formation.

Under special circumstances, substances preventing the action of enzymes are to be met with. An interesting one is that present in *intestinal worms*, protecting them from the action of trypsin. The properties of this substance were especially investigated by Hamill (1906) and it was found to be soluble in 85 per cent. alcohol, not destroyed by boiling in neutral or acid solution, but readily in alkaline solution. It is not a colloid. When added to a tryptic digest, it is found to disappear slowly, so that ultimately the enzyme recovers its full activity and is, therefore, merely temporarily paralysed. Its disappearance in the alkaline digest is natural, owing to its sensibility to alkali. As will be seen, this substance has none of the characteristics of Ehrlich's antibodies.

The behaviour of *raw serum* or egg-white to trypsin is peculiar. If the curves on p. 129 of my monograph (1919, 1) be referred to, it will be noticed that the action on raw egg-white starts slowly but becomes more rapid until it ultimately reaches the same point as when the substrate had been previously boiled. This may be due to the presence of some inhibitory substance similar to that of the intestinal parasites, or perhaps to the adsorption of the enzyme by the protein, which is itself a difficult one for attack; as this protein is slowly attacked, the enzyme is set at liberty, so that it is available for the further conversion of the easily attacked proteoses resulting from the initial hydrolysis of the protein.

Concentration of Substrate.—According to the law of mass action, it is to be expected that the rate of change in an enzyme reaction would be directly proportional to the concentration of the substrate. This is so, in the main, so long as the concentration does not exceed a certain value, which differs in individual cases. In that of caseinogen, for instance, below 5 per cent. the rate is proportional to the concentration, although not in simple linear ratio; above 5 per cent., the rate continues about the same up to 8 per cent., but in 10 per cent. solution it is rather less than in one of 8 per cent. There appear to be two factors concerned. The rate of a reaction in such colloidal heterogeneous systems, as we have seen, is determined by the amount of the adsorption compound between enzyme and substrate in existence at any given time. Remembering further what we have learned with regard to adsorption in general, we see that, at a certain

concentration of substrate, the active surface available will be "saturated," so that further increase in concentration will not result in more adsorption and therefore in no increase in the rate of the reaction. If the "concentration" of the active surface is increased relatively to the substrate, there will be increase until this surface is saturated.

This circumstance, however, seems capable of explaining only the fact of *equality* of rate above certain relative proportions of enzyme and substrate, and is well illustrated by the following two experiments by E. F. Armstrong (1904, 1, p. 508). A very small amount of lactase acted on different concentrations of lactose for forty-six hours; it was found that the amount hydrolysed was the same in all, although the reaction was by no means at an end, thus:—

Lactose.	Amount Hydrolysed.
10 per cent.	2·22
20 ,,	2·18
30 ,,	2·21

When the proportion of enzyme to substrate was large, a different result was obtained:—

Lactose Per Cent.	Change in Three Hours.	Velocity Constant.
1·0	0·185	0·0296
0·5	0·098	0·0298
0·2	0·0416	0·0337

The amount of hydrolysis is in direct ratio to the concentration of the substrate and the velocity constant is practically identical in all.

Another factor which comes into play in such cases as proteins or glycerol is *viscosity*, which, as we have seen, retards the access of substrate to enzyme. The fact that gelatine shows actual retardation above a certain concentration and in a more marked degree than does caseinogen, supports this view, since gelatine forms solutions of a higher degree of viscosity than those of caseinogen. The following numbers show the change of electrical conductivity in twenty-five minutes in solutions of gelatine of different concentrations:—

10 per cent.	130 reciprocal megohms.
8 ,,	170 ,, ,,
4 ,,	240 ,, ,,
2 ,,	280 ,, ,,

We see that there is a progressive increase in the rate as the solution is more dilute. Similar facts apply to the synthesis of glycerol-glucoside by emulsin; the *rate* is diminished when the glycerol present exceeds about 65 per cent. It is important to note, however, that in this case, where it is possible to test the effect on the total amount of products when the reaction has attained equilibrium, this final amount is found to be in direct ratio to the concentration of the substrate, although the higher the viscosity, the longer the time taken to reach equilibrium.

Effect of Temperature.—Like all processes, the action of enzymes is increased in rate by rise of temperature, in some cases very considerably, more than trebled by a rise of 10°. The fact indicates that the controlling factor of this particular kind of heterogeneous reaction is the chemical reaction proper, since both diffusion and adsorption, as physical processes, have a low temperature coefficient.

As the temperature is raised, it is found that, above a particular temperature, the rate begins to fall off, and at a further rise of temperature all effect is abolished. The temperature at which the maximum rate is shown has been called the *optimum temperature.*

It is merely due to the fact that enzymes are injured more or less rapidly by rise of temperature, and the optimum temperature is that at which the acceleration due to rise of temperature is in greatest excess over the simultaneous destruction of the enzyme. The process has been worked out by Frost Blackman (1905) and a complete explanation given. Attention should also be directed to the time factor in this connection. The lethal effect of raised temperature is not a sudden thing, so that the slowing of the reaction will be more and more apparent the longer the time that has elapsed since the commencement of the exposure to a

particular temperature. Neither the optimum nor the lethal temperature is a fixed point; a short exposure to a high temperature may not kill an organism, while a longer exposure to a rather lower one may be fatal.

Some of the earlier workers with enzymes seem to have regarded the optimum temperature of enzyme action as something mysterious, even indicating "vital action." We see that it is merely the expression of the sensitiveness of the colloidal arrangements of the enzyme system to rise of temperature, and is not confined to enzymes, but may be shown by inorganic colloids.

FIG. 85. INTERIOR OF VAN'T HOFF'S PRIVATE ROOM IN THE OLD LABORATORY AT AMSTERDAM (1877-1891).—On the left of the photograph is a window, in front of which stands a table. This table was van't Hoff's chief workplace.

(Reproduced by the kindness of Prof. Ernst Cohen of the van't Hoff Laboratory, Utrecht.)

There is one practical point in connection with the great acceleration of enzyme action by rise of temperature. It is often necessary to stop a reaction at a particular stage; this must not be done, if any accuracy is required, by raising the sample to the boiling point. However quickly this can be done in practice, the enzyme is not immediately destroyed, and during its short life, it acts with great energy, owing to the considerable rise in temperature. If dilution is permissible, the sample may be allowed to fall, drop by drop, into boiling water. Better, if the addition of chemical substances is immaterial, as is usually the case, mercuric nitrate may be added, which immediately precipitates and destroys the enzyme.

EQUILIBRIUM AND REVERSIBILITY

Although the great majority of the reactions catalysed by enzymes are
to be reversible, it is a matter of, at any rate, great theoretical interest to
how far it is justifiable to regard all reactions as reversible. Accor
J. J. Thomson (1888, p. 281), if we take the view that the properties of
in motion are sufficient to account for all physical phenomena, irre
processes, such as those apparently made so by frictional resistance,
capable of explanation as the combined effect of changes, all of which ar
selves reversible. "It follows that, if we could only control the phen
in all its details, it would be reversible; so that, as was pointed out by
the irreversibility of any system is due to the limitation of our po
manipulation. The reason why we cannot reverse every process is bec
only possess the power of dealing with the molecules *en masse* and not indiv

FIG. 86. EXTERIOR OF VAN'T HOFF'S OLD LABORATORY AT AMSTERDAM.

(Reproduced by the kindness of Prof. Ernst Cohen
of the van't Hoff Laboratory, Utrecht.)

while the reversal of some processes would require the reversal of the m
each individual molecule."

Nernst, also (1911, p. 442), states that reactions cannot be divid
reversible and non-reversible. "There can be no doubt that by suitable
ment of the conditions of the experiment, it would be possible to make a
take place, now in one direction, now in the opposite, that is, *in princip
reaction is reversible.*" One of the most obvious of these conditions refe
is that of temperature, as in the well-known case of the dissociation of am
chloride.

It appears justifiable, then, to regard all reactions from this point
although, under ordinary conditions, the position of equilibrium may be
the state of complete change in one direction that the reaction seems to ha
entirely in one direction.

When a reaction is known to be reversible, it is customary to use the sign sugg
van't Hoff (1884, p. 5), namely, two arrows pointing in opposite directions:—

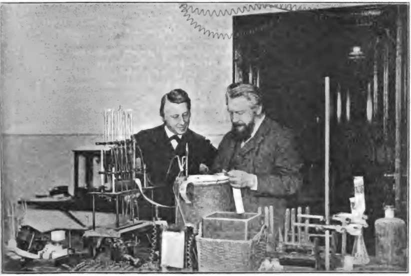
$$\text{acid} + \text{alcohol} \leftrightarrows \text{ester} + \text{water},$$

or more conveniently, by the modification of this suggested by H. Marshall (1902): $\rightleftharpoons$

We owe to van't Hoff (1884) the systematic investigation of the laws
velocity of reaction and of equilibrium. It may be of interest to the re

see a photograph of the laboratory in which he worked from 1877 to 1887, a period of time in which so much of fundamental importance was produced. The photograph of van't Hoff's workroom, reproduced in Fig. 85, and that of the exterior of the laboratory in Fig. 86 are taken from the book by Jorrisen and Reicher (1912). Fig. 87 is an interesting picture of Ostwald and van't Hoff.

We have already had occasion to refer to some facts concerning the *reversibility of enzyme action*. We saw that the equilibrium point may be in very various positions according to the relative rate, as determined by the chemical difficulty, of the two opposing reactions. We have seen how, at this equilibrium point, the two opposing reactions are to be regarded as still proceeding, but at equal rates. The reaction with the slower natural rate is compensated for by the greater active mass at the state of equilibrium. It is to be remembered that the actual position of equilibrium is decided by the relative values of the two velocity constants. The ratio of these constants is obviously also a constant and is known as the *equilibrium constant*. We note further that the equilibrium position can be defined in two ways, either from the dynamical point of view, as above, by the ratio of the two velocity constants, or, from the statical point of view, as the ratio of the relative masses of the components. It is interesting to find that the equilibrium constant in the lipase reaction of Dietz, given at the commencement of this chapter, is found experimentally to be the same when calculated in both ways.



J H van't Hoff. W Ostwald

FIG. 87. VAN'T HOFF AND OSTWALD IN OSTWALD'S LABORATORY AT LEIPZIG.

(Reproduced by the kindness of Prof. Ernst Cohen of the van't Hoff Laboratory, Utrecht.)

Although the equilibrium position can be defined by the relative masses of

the components, it is not to be supposed that it is a statical one. The two reactions are still proceeding, the various molecules are continually changing their partners, but, during the same time, the number of changes in the one direction is equal to that in the opposite one.

This conception of a *dynamical equilibrium* is of great importance, not only in chemistry, but also in the physiology of the cell. The idea seems to have been first clearly expressed by A. W. Williamson (1850).

Before proceeding further, some additional remarks on the law of *mass action*, especially on its history, are required. Before the time of Berthollet (1799), it was generally held that the course of chemical action had nothing to do with the quantity of reacting matter. This chemist, however, pointed out how the reaction—

$$CaCl_2 + Na_2CO_3 = CaCO_3 + 2NaCl,$$

was reversed, on the shores of certain Egyptian lakes, by the presence of great excess of calcium carbonate, so that the deposits of sodium carbonate were thus to be accounted for. As he says, "an excess of quantity can compensate for a weakness of affinity," and "the result of a chemical reaction depends not simply on the strength of the affinities, but also on the amount of the active reagents" (p. 5 of the reprint in Ostwald's "Klassiker"). This point of view was not accepted for more than half a century. In 1850 Wilhelmy applied mass action in a quantitative manner to the hydrolysis of cane-sugar by acid, and established the fact that the rate of action at any moment is proportional to the amount of substance undergoing change. Harcourt and Esson in 1856 obtained similar results, but it is the great service of Guldberg and Waage (1864) to have formulated and applied the idea in its full significance, and in a clear and systematic manner. Nevertheless, their work remained for a long time unknown, so that the law of mass action was developed independently by Jellet in 1873, and by van't Hoff in 1877.

To avoid possible confusion, it should be clearly understood that the masses spoken of are *concentrations*, that is, mass in unit volume. Taking again the kinetic point of view, we can see at once that it would not double the number of effective collisions if we doubled the mass and the volume at the same time; there would still be only the same possibility of collision. We must ensure the possibility of doubling the number of collisions by doubling the number of molecules in the same space.

Remembering that the *composition* of a system in equilibrium is determined by the relative *rates* of two opposing reactions, we see how the law of mass action is the basis, not only of chemical dynamics but also of chemical statics.

Passing on to consider its application to the action of enzymes, let us see first what is the effect of changing the concentration of one component of a reversible reaction in equilibrium. Taking the familiar ester system, the rate of hydrolysis is in proportion to the product of the concentrations of the ester and the water, that is:—

$$v_1 = k_1 \times (\text{ester}) \times (\text{water}),$$

that of the synthetic reaction is:—

$$v_2 = k_2 \times (\text{alcohol}) \times (\text{acid}),$$

using brackets as usual to express concentrations, and k_1 and k_2 are the two velocity constants. Then, in equilibrium:—

$$k_1(\text{ester})(\text{water}) = k_2(\text{alcohol})(\text{acid}), \text{ or}$$

$$\frac{k_1}{k_2} = \frac{(\text{alcohol})(\text{acid})}{(\text{ester})(\text{water})}, \text{ and, as we saw before,}$$

$$\frac{k_1}{k_2} = \text{K, the equilibrium constant.}$$

Put in this form, we see that if we increase one component, the result must be to decrease its fellow, since the value of the fraction must remain unaltered. Suppose we increase water, the value of the fraction can only be kept constant

either by increasing (alcohol) (acid) or by decreasing (ester). In point of fact, of course, the two are identical, since one cannot take place without the other. The result of excess of water should be, therefore, to increase the hydrolytic reaction of the system, as found by experiment.

The conclusion to be drawn from this fact is that, in order to obtain much indication of the synthetic aspect of enzyme action, the concentration of water must be diminished as far as possible (Fig. 80, page 300).

In the living cells, where synthetic processes readily take place, it seems that there must be some very effective means of doing this, perhaps by surface condensation or imbibition on the part of colloids. But we have as yet no very clear idea of the mechanism.

As has been pointed out above, certain synthetic reactions proceed but very slowly, even in maximum concentration of the reagents, on account of their chemical nature itself. But, in the dynamic and heterogeneous systems of the cell, this small amount of synthesis must not be undervalued. Suppose that, as soon as equilibrium is established, the synthetic products are removed in some way. More will be formed in order to re-establish the stable condition and, in this manner, the process may be continuous, so that a quite appreciable degree of synthesis may take place in a short time, depending on the extent to which the reaction is accelerated by an enzyme. The removal may be effected in several ways. The product may be washed away by the blood current to some other part of the organism, it may be deposited in the form of a separate phase, such as starch, glycogen, or fat, or it may be immediately used up in an independent chemical reaction.

A particular enzyme in a cell, for example amylase in the liver, will, under low concentrations of glucose in the blood, hydrolyse the glycogen stored in the cell; while, in higher concentrations of glucose, glycogen will be synthesised and, as it is stored in an insoluble form, the process can go on to a considerable extent. This possibility has been pointed out by Croft Hill (1898).

The hydrolysis and loss of starch from germinating seeds is regulated by the growing plant. If the embryo is removed, the starch ceases to be hydrolysed. This is obviously a case of equilibrium of the kind just referred to, since Pfeffer and Hansteen (1893) have shown that, if the embryo of maize or barley be replaced by a little column of plaster of Paris, the disappearance of starch can be stopped or set going again according as the end of the plaster column is immersed in a tiny drop of water or in a large quantity. In the former case, the products of hydrolysis are not removed, so that the reaction soon comes to its equilibrium position. In the latter case, they are removed by diffusion as fast as they are formed, so that their concentration is maintained permanently low and no equilibrium is reached.

The reader is referred to the chapter on the reversibility of enzyme action in my monograph (1919, 1) for the numerous cases in which direct evidence of synthesis by enzymes has been observed. The fact must be again emphasised that there is no necessity for the assumption of special synthesising enzymes, and that all evidence that has been brought forward to show their existence has been shown to be capable of other explanations (Bayliss, 1913). If enzymes are catalysts and if the reactions are reversible ones, enzymes must accelerate both the hydrolytic and the synthetic aspects, unless they carry the reaction to completion in one direction, whatever the conditions present.

It was mentioned incidentally at the beginning of the present chapter that the actual position of equilibrium is frequently found to be somewhat different under the action of enzymes from that under acids. This fact seems to have caused some difficulty. But there are one or two considerations, of interest in themselves, which should, I think, lessen or remove the difficulty. The difficulty itself would be much more serious if these enzyme changes were associated with any considerable heat change, since, in that case, energy would have to be supplied by the enzyme or from some other source. But since the active enzyme is always present in minute amount compared with that of the substrate it does not seem possible that it could supply any appreciable amount of energy,

either by chemical or physical change. Hydrolytic actions, and it is in these that the question arises, are practically thermo-neutral, as pointed out by van't Hoff (1909, p. 1075); the heat change is very small; in the conversion of one gram-molecule of methyl acetate to one of alcohol and one of acid, only −0·9 large calories, thus:—

Heat of combustion of methyl alcohol	170·6
„ „ acetic acid	61·7
	232·3
„ „ methyl acetate	233·2
Difference	−0·9

In other words, only 0·38 per cent. of the heat of combustion of the ester. The small amount of energy required to change the equilibrium position, in the case of ethyl butyrate, in the experiments of Dietz from 85·5 per cent. of ester, when acid was the catalyst, to that of 75 per cent. when lipase was used, might quite conceivably, as Herzog (1910, p. 196) points out, be obtained from surface or volume energy of some kind. In fact, the difference between catalysis by acid and that by enzyme consists essentially in the circumstance that the former results in an equilibrium in a homogeneous system, the latter in one in a heterogeneous system.

If this shifting of the equilibrium position is due to the supply of energy from some component of the system, it seems difficult to suppose that it can be from the enzyme itself; if so, the equilibrium should not be the same with different amounts of enzyme, whereas we have seen that, in experiments in which it was certain that equilibrium was really attained, the concentration of enzyme played no part in the equilibrium. It may be, nevertheless, that the amount of energy required is so small that it was supplied by the smallest concentration used.

A subsidiary point is worth mention here. We have seen that lipase is increased in its activity by bile salts, and the question arises, does this increased activity affect both the components of the reversible reaction? It has been shown by Hamsik (1910) that it does; the position of equilibrium is unaffected. The fact serves to confirm the view taken of the mode of action of the co-enzyme in this case, namely, that it increases the active surface of the enzyme.

Bourquelot et Bridel (1914) made the interesting observation that, if maltase and emulsin together act on glucose and alcohol, so that a mixture of the α- and β-glucosides is formed, the same composition of the system in equilibrium is attained, whatever the relative amount or the order in which the enzymes are added. There must, therefore, be a conversion of the one glucoside into the other, presumably after previous hydrolysis.

MODE OF ACTION

The manner in which catalysts act appears to be of more than one kind, so that no satisfactory general statement can be made.

Formation of Intermediate Compounds.—In one case, that of the acceleration of the reaction between hydriodic acid and hydrogen peroxide by molybdic acid, this stage of intermediate combination has been satisfactorily shown by Brode (1901) to be passed through. A series of permolybdic acids is formed by the action of hydrogen peroxide on the catalyst; these are formed with great rapidity and, when formed, they react also with great rapidity on hydriodic acid, with separation of iodine and return of the catalyst to its original form of molybdic acid. Both these reactions together occur at a greater rate than the original uncatalysed reaction between hydriodic acid and hydrogen peroxide, so that the criterion of Ostwald (1899, p. 517) as to the conditions to be satisfied for such an explanation to be admissible are present.

It must be admitted, however, that this case of catalysis with the formation of an intermediate compound of a chemical nature appears to be an exceptional one. In some cases, indeed, as in the catalysis of methyl acetate by hydrochloric acid, it is found that the reaction by way of methyl chloride takes *longer* than the spontaneous actual one, so that this obvious intermediate compound is excluded.

Adsorption.—It was suggested above that the increased concentration produced by adsorption might perhaps be sufficient to account for the greater rate of reaction. But it scarcely seems possible to explain the existence of such

a variety of enzymes on this hypothesis alone, although one must not be too hasty in making such a statement until more is known as to the nature of adsorption in its manifold aspects.

With respect to the numerous theories of catalysis that have been suggested, the reader may consult the work of Mellor (1904, chapter x.).

Before proceeding to a discussion of what we know as to the mode of action of enzymes, a brief description of their physical and chemical properties, as far as they are known, is requisite.

Physical Properties of Enzymes.—They are all in the colloidal state in solution. They do not diffuse through thick parchment paper, but, as samples of this paper vary in the dimensions of their pores, it may be found that an enzyme in a highly dispersed condition may diffuse slowly through some papers. This was the case with the amylase of Fraenkel and Hamburg (1906).

Their destruction by heat has been referred to above.

As colloids, they have an electrical charge, varying with the electrolytes present with them. This charge appears to play some part in the mechanism of their action, as will be seen presently.

There is indirect evidence that many, if not all, are optically active.

The Chemical Nature of Enzymes.—It is obvious that great practical difficulty exists in the investigation of this subject, owing to the minute amounts of these intensely active substances which we have at our disposal. It was thought at one time that they had the composition of proteins, but, as preparations were made of greater purity, it was found that the protein reactions disappeared more and more, although the preparation gained in activity. Moreover, according to Beijerinck, they are incapable of serving as nitrogen food for bacteria or yeast. It is probable that, like inorganic catalysts, they are of very varied chemical nature, but what this is cannot as yet be stated definitely in respect of any one of them.

It is possible that they are not single chemical individuals, but complex systems, as was suggested by Bertrand some years ago. In an address to the French Association for the Advancement of Science in 1909, the theory is stated as follows:—One of the constituents of the system is capable, on its own account, of producing the reaction in question to a slight degree, but requires the presence of another substance, inactive in itself, before its activity becomes appreciable. The former is, according to the case, some such substance as acid, alkali, calcium or manganese salt, etc. The latter is a more complex substance, often similar to egg-white, colloidal in character. This view is similar to that stated by von Wittich (1872, p. 469) as regards pepsin, which is held merely to intensify the action of hydrochloric acid. It is not quite clear, however, whether von Wittich intended to make the general statement that all enzyme actions are of this nature, although it seems implied. This view receives support also from the facts connected with the "artificial laccase" prepared by Dony-Hénault (1908, p. 151), in which the active agent is colloidal manganese hydroxide, but protected from aggregation by the presence of a "stable" colloid, gum arabic.

At this point I feel bound to make a slight protest against Bunge's gibe at physiologists (1907, p. 241), in which he says that "the less a physiologist knows about chemistry, the greater is he inclined to work at the most difficult chemical subjects—the proteins and ferments." If the chemistry to which reference is made here is pure statical, structural, organic chemistry, as would appear, it is a remarkable fact that such a mode of attack has taught us practically nothing about the nature of enzymes, and has only led to the multiplication of names, on which Bunge himself justifiably throws contempt as "a drag and a brake to science." It is only since the question has been attacked from the kinetical standpoint of physical and colloidal chemistry that we are beginning to see light. It is, of course, far from my intention to undervalue the work of organic chemistry as one of the helps to the comprehension of our difficult problems, as must be apparent from the previous pages of the present book, and would be of self-evident absurdity; but, in view of opinions sometimes expressed, it is necessary to point out that there are other bodies of doctrine of equal importance in the study of physiology.

Enzymes Act at their Surfaces.—The clearest direct proof of this fact is that emulsin, lipase, urease, and trypsin exert their activity in alcoholic media in which they are completely insoluble, and can be filtered off (Bayliss, 1915). In such

cases, where the enzyme is not uniformly distributed, rate of *diffusion* must play a part in the first stage of the particular heterogeneous reaction, as, in fact, is found by experiment, since shaking such systems accelerates the rate of change. When the enzyme is in colloidal solution, although it forms a separate phase, it is comparatively uniformly distributed, so that the diffusion distances are very small and we can, with caution, apply the formulæ of velocity of reactions developed for homogeneous systems. *Adsorption* of substrate on the surface of the enzyme phase is the next stage, as we saw in describing heterogeneous reactions in general. This probably takes place with great rapidity as soon as the components are sufficiently near together. *Chemical reaction* follows; but, under conditions in which it takes place slowly, cold for example, it is possible to separate the actual adsorption compound of enzyme and substrate. The "compound" of starch and amylase has been prepared by Starkenstein (1910) and by Philoche (1908, p. 393), that of fibrin and pepsin by von Wittich (1872, p. 444), those of trypsin with starch, caseinogen, and charcoal, and of amylase with caseinogen by myself (1911, 1). It will be noted that it is not necessary that the substrate should be one on which the enzyme acts in order that adsorption may take place.

A further point of interest is that electrolytes behave in this process in the same way as that in which they behave in what we have called above "electrical adsorption" (page 58), as shown by myself in the case of trypsin (1911, 1). If the enzyme and the substrate are both negatively charged, a certain obstacle to adsorption exists, since, if it took place, it would increase the electrical energy of the surface. If a bivalent ion, say $Ca^{\cdot\cdot}$, is present, the charge on the surface is reversed and adsorption facilitated. In this way the favourable action of electrolytes in many cases can be explained.

An *intermediate compound* of a chemical nature between enzyme and adsorbed substrate has not been shown to be formed.

The work of Wöhler, Plüddemann, and Wöhler (1908) is of some importance in this connection. Their investigations concern the catalytic action of various oxides and of platinum on the oxidation of SO_2 in the manufacture of sulphuric acid. They show that any sulphites or oxides of the ordinary kind are inadmissible as intermediate chemical compounds between catalyst and substrate. If such a compound is to be assumed, it must be an endothermic one, such as a peroxide. They regard their experiments as more favourable to the theory of acceleration by increased concentration due to adsorption, but do not consider them as definitely deciding the question.

The possibility of increased chemical potential brought about by molecular forces in the act of concentration on the surface, as pointed out by Hardy, must not be forgotten.

If, as we assume, the natural state of equilibrium is brought about rapidly by increased concentration on the surface, it follows, as Prof. Hopkins reminds me, that the various components of the system must be adsorbed in the same proportion as they exist in the body of the solution. We have, as yet, insufficient evidence on the question. It may be that the explanation of the different equilibrium position under acid and enzyme may be found here. The mechanism of heterogeneous catalysis requires much further investigation.

There is the possibility that $H^{\cdot}$ and OH' ions may be also adsorbed and assist in the process, somewhat as in the theory of Bertrand (p. 325). Mellanby and Woolley (1915, p. 258) hold that pancreatic amylase "associates" $H^{\cdot}$ ions with itself and that its activity is determined thereby.

The following illustration may assist the reader in understanding the facts of heterogeneous reactions. I must apologise for its apparently trivial nature. Imagine a number of snails in the neighbourhood of a strawberry. As soon as a snail, in the course of its wanderings, becomes sensible of the presence of the food, it proceeds towards it. This is the preliminary diffusion, and would perhaps be more like the real kinetic process if we suppose that the snail was insensible of the existence of the strawberry until it accidentally came into contact with it. The next stage, that of adsorption, follows rapidly as the animal attaches itself to the fruit. If nothing more happens, there is no chemical reaction. The final, chemical stage is the devouring of the food and its subsequent hydrolysis. It is obvious that the rate of this final stage is proportional to the number of snails "adsorbed." It will also be noted that it is not in linear ratio to the number at work. The more there are, the more they interfere with one another, and, when the strawberry is completely covered, the advent of more snails will not further increase the rate of disappearance, since the newcomers cannot get at the fruit. The strawberry here corresponds to the enzyme; we may imagine that, instead of the fruit, we

have a powerful chemical substance which induces the disintegration of the snails, representing the substrate, which are adsorbed on its surface.

The exponential ratio of the concentration of the enzyme to its activity receives a satisfactory explanation on this adsorption theory, as will be plain from the above illustration.

On the other hand, it seems that we must either attribute some special properties to the enzyme surface itself, which may be of the nature of configuration, chemical or physical, or else we must suppose the formation of an intermediate chemical compound between enzyme and substrate, to be afterwards broken up into enzyme and products. The case of the relation between the α- and β-glucosides to maltase and emulsin will serve to show what is meant here. The α-glucosides are scarcely acted on at all by emulsin, perhaps not at all, but rapidly by maltase, and vice versa with regard to the β-glucosides. Now it does not seem possible that any ordinary surface could distinguish to such a degree between the properties of two substances so nearly alike as the α- and β-glucosides of methyl are. At the same time, apart from their optical isomerism, they have certain other differences, solubility for example. As was remarked before, until we know more as to the possibilities of adsorption, it would be rash to be dogmatic on the question.

As to the configuration of the surface, it is quite conceivable that a particular pattern, so to speak, may allow closer approximation of reacting molecules than another pattern does. As a very rough illustration, a surface beset with projecting spikes would not allow so close an approximation of a flat surface as would another flat surface. We must be careful, however, not to be misled by too statical a conception of the phenomena. Moreover, there may be true chemical combination with the actual *chemical substance of the surface* of a colloidal aggregate, without the phenomena losing their characteristic adsorption nature. See also Barger and W. W. Starling (1915).

For further discussion of the nature of enzyme action, the reader may consult the author's monograph (1919, Chapter VII.). The process is evidently a special case of catalysis in heterogeneous systems, and the papers by Bancroft (1918) should be read. In such systems the rate of reaction is conditioned by the amount of reacting substances adsorbed on the surface of the catalyst. It must not be forgotten, however, that the physical properties of the surface on which adsorption depends are the result of the chemical nature of this surface. Whether adsorption is followed by chemical combination with the catalytic material, or whether the close approximation of the adsorbed substances is sufficient, cannot as yet be regarded as definitely known. At the same time, it is striking how more and more of the facts are becoming explicable on the latter basis. It appears probable, moreover, that even when there is chemical combination with the catalysts, this is not necessarily an essential factor in the process and may be a disturbing collateral reaction. There is need of much further work on the question, especially with respect to the effect on the final equilibrium of different relative adsorption of the constituents of the liquid phase. The papers by Hilditch and Armstrong (1919) contain interesting facts relating to the equilibrium in the case of metallic catalysts and to their synthetic activity.

ZYMOGENS

When enzymes are produced by cells, it is plain that they must pass through preliminary stages, and it seems that what are called "zymogens" constitute a stage of this kind. Sometimes we find the enzymes secreted to the exterior in the inactive form; the trypsinogen of the pancreatic juice is such a case; it requires the action of another enzyme, enterokinase, to convert into active trypsin. Details of the phenomenon may be found in my monograph (1919, 1) and to some extent in the following chapter of this book.

We must note the difference between a zymogen and an enzyme which is inactive on account of the want of its co-enzyme. The conversion of a zymogen into an enzyme cannot be reversed by any process at present known to us, whereas the co-enzyme can be added or removed at will.

PRODUCTION OF ENZYMES

There appears to be some evidence that enzymes may make their appearance in response to the presence of an appropriate stimulus, or rather substrate. Thus Duclaux (1899) stated that *Penicillium glaucum*, grown on different media, produced enzymes which hydrolysed these media, enzymes which were absent in other cases.

A significant point with regard to the nature of enzymes is to be found in their occurrence in situations where they have never had the opportunity, in the course of evolution, of meeting with their special substrates, lactase in the almond, for example. If this lactase is a selective enzyme acting only on lactose it must have been produced, accidentally, as it were, as a by-product of metabolism. Otherwise it must be regarded merely as an incidental property of emulsin.

The appearance of enzymes in the blood in response to injection of proteins or carbohydrates, "protective enzymes" (Abderhalden und Kapfberger, 1910), requires further investigation. They are not specific, that is, a particular sugar may set free the enzyme which hydrolyses it or another in addition. They are probably set free from some situation in the organism. The production of an enzyme, not found somewhere in the organism, has not been shown to occur.

SPECIFICITY

We are quite justified in speaking of the relation between the α- and β-glucosides and maltase and emulsin as a "specific" one, although the difference may be merely quantitative, as we shall see presently. There are, however, many degrees of specificity; emulsin acts on a great variety of glucosides, trypsin on all proteins, while invertase is said to have no action on any substance but cane-sugar. This last fact places a difficulty in the way of accepting Bertrand's hypothesis, at least in its simplest form. Since, if invertase merely activates acid, it should be capable of hydrolysing maltose and lactose as well as saccharose.

But it seems to me that the practice of some investigators in assuming a separate enzyme for every substrate acted upon is not warranted by the facts. When we say that there is a salicinase in what is usually called emulsin, if we mean anything more than that emulsin hydrolyses salicin, we are going beyond what is justified by the experimental evidence. It is true that, under some conditions, extracts containing emulsin may act more powerfully on salicin than upon some other glucoside, while other extracts may act better on the latter; but it has not been shown that this is due to anything other than different conditions. It is to be remembered that even acid will hydrolyse some glucosides much more readily than others. Until a separate enzyme is prepared which acts on no other substrate but salicin, under any conditions, the name salicinase should not be used. At the present time it would be more profitable to devote attention to the various ways in which the rate of action of an enzyme on various substrates can be modified by change of conditions (Armstrong and Horton, 1912).

The multiplication of names may even be mischievous in leading to the belief that new knowledge has been obtained when a phenomenon is described by a name derived from a classical tongue instead of in English.

There is risk, for example, that when we say that the injection of a foreign protein causes the production of a "precipitin" for the protein, we may imagine that this "precipitin" has been shown to be a definite chemical individual, instead of a mere description of the fact that a precipitate is formed. The "side-chain theory" of Ehrlich, great as has been its use in suggesting problems for investigation, is, at present, overburdened with multitudes of names, which consist, for the most part, merely of descriptions of the phenomena, although they suggest actual substances. There are many signs that one or two simple explanations, on the basis of colloidal chemistry, will be found to put an end to most of these names.

One is tempted, indeed, to make a well-known quotation from Molière (1673). The reader will remember that in the ballet of "Le Malade Imaginaire," which ballet is a satire on medical examinations, one of the medical students sings (tome v. p. 308 of Hachette's edition):—

"Mihi a docto doctore
Domandatur causam et rationem quare
Opium facit dormire.
A quoi respondeo,
Quia est in eo
Virtus dormitiva,
Cujus est natura
Sensus assoupire."

Which I may venture to translate thus:—

"The learned doctor asks me
The cause and reason why
Opium sends to sleep.
To him I make reply,
Because there is in it
A virtue dormitive,
The nature of which is
The senses to allay."

For this profound answer the candidate receives his diploma with acclamation, together with his licence to "kill and to cure."

Incidentally, I would call attention to the fact that this play was the last written by its author, although it is regarded by many as his best work. This fact may be commended to the attention of those who wish to prevent men at any particular age from taking part in the work of the world.

Galileo was over seventy years of age when he wrote one of his best works, and thought out, amongst other things, the application of the pendulum to the regulation of clocks. In one of Leeuwenhoek's letters we find the words: "A certain gentleman, who was with me some months ago, intreated me to go on in making observations, adding that the fruit which ripen'd in autumn was the most lasting. This is now the autumn of my life, I being arrived at the age of 88¾ years" (H. G. Plimmer, 1913, p. 135). Many other instances might be given, from the sphere of "action" as well as that of scientific discovery.

To return to our theme. As already remarked there are various facts which are calculated to give us pause before accepting, as an article of faith, the doctrine of the perfect specificity of enzymes. They will be found in my monograph (1919, 1, pp. 135-141) by those interested. There are one or two points of general interest which may be mentioned here.

Dakin (1904) found that, when lipase was used for hydrolysis of the optically inactive mixture of the two mandelic ethyl esters, one of the isomers was hydrolysed more rapidly than the other, although finally both were completely decomposed. This was brought into relation with the probable optical activity of the enzyme, so that the "compounds" of this with the two forms of the ester would not be symmetrical, and would therefore decompose at an unequal rate. Similar facts are described by Fajans (1910) with regard to the decomposition of the two camphor-carboxylic acids by optically active bases, acting as catalysts, and, as regards synthesis, by Rosenthaler (1909) in the case of emulsin forming benzaldehyde-cyanhydrol (Bredig und Fiske, 1912). We have seen that there are many cases known where living organisms consume preferably the one isomer, but when this has disappeared, the opposite one is also attacked. Dox and Neidig (1912) show how extracts of Aspergillus hydrolyse both α- and β-methyl glucosides, but at an unequal rate. There are other cases known where extracts of tissues, which were originally supposed to contain only one kind of enzyme, say maltase, have been found to act, slowly, on the opposite isomer. It seems to be simpler to regard these as due to a slow action of the same enzyme on both isomers than as due to the presence in traces of another enzyme, especially when this other enzyme is one which, under natural conditions, would never have had any opportunity of action. Fajans (1910) has shown in detail how much more satisfactorily the various experimental data can be explained on this hypothesis.

The results of Erlenmeyer, mentioned above (page 285), show the possibility of an optically active enzyme acting more rapidly on the appropriate component of a racemic mixture if an indifferent optically active substance is present. Such a process of conversion appears to be independent of chemical combination in the usual sense.

SUMMARY

There are a large number of reactions which proceed by themselves very slowly, or sometimes, apparently, not at all, but which can be enormously accelerated by the presence of small amounts of various foreign substances.

The characteristic property of such accelerating agents, known as "catalysts," is that they do not form part of the system in its final equilibrium, and either appear at the end in their original form or, in some cases, are partially destroyed or removed from the sphere of action in the form of constituents of some subsidiary reaction.

When the system is one that reaches a definite equilibrium under the conditions of the experiment, the position of this equilibrium is unaffected by the presence or the amount of the catalyst, which merely hastens the time taken for the process, and this in proportion to its concentration.

Two important things are shown by this fact, namely, that the catalyst does not supply or remove energy from the system, and that it accelerates both the hydrolytic and synthetic components of a reversible reaction.

In living organisms there are a large number of substances which behave like catalysts, and are known as "enzymes."

They are extremely active, and explain the occurrence in the organism of reactions which require, in the laboratory, powerful reagents and high temperatures. Lactose is hydrolysed by both hydrochloric acid and by an enzyme, lactase; but weight for weight, the latter is, at least, five thousand times as powerful as the acid.

These enzymes are all in the colloidal state, that is, they form a separate phase of the heterogeneous system. Their action is exerted on their surface, and is controlled by the amount of reagents adsorbed.

The substances acted on by enzymes are usually called "substrates."

The heterogeneous nature of the systems in which enzymic reactions occur is, in all probability, the reason why the equilibrium position is not quite the same under the action of enzyme and under that of acid. But, owing to the almost complete thermo-neutrality of the hydrolytic reactions in question, an extremely small amount of energy is all that is necessary to change the equilibrium to the extent found.

Whenever the equilibrium position under the action of an enzyme, or other catalyst, is anywhere except at complete change, either in the direction of hydrolysis or synthesis, the enzyme must accelerate *both* reactions, although not necessarily to an equal extent. This relative degree of acceleration depends on the respective chemical difficulty of the two reactions of hydrolysis and synthesis.

Enzymes, therefore, bring about synthesis as well as hydrolysis.

There is no sufficient evidence that the enzyme forms a constituent of the final chemical equilibrium.

Since the essential property of an enzyme or catalyst is to change the rate at which a reaction proceeds, a discussion of the formulæ of velocity of reactions, deduced from the law of mass action, so far as applicable to the case, is introduced into the text.

When the velocity constant of an enzymic reaction is calculated by the appropriate formula, it is found that it suffers, as a rule, considerable diminution as the reaction progresses. This means that the activity of the enzyme is decreasing. In some cases there is a spontaneous destruction of the enzyme, in others it is merely temporarily paralysed by some products of the reaction. In the latter case, the most frequent cause is change of the hydrogen ion concentration to a point above or below the optimal one. Enzymes, in fact, are very sensitive to such changes.

Some evidence has been brought forward to show that there is a special chemical affinity on the part of the enzyme for the substrate or for some constituent of it; but, at present, the evidence is not very convincing.

The relation between the concentration of an enzyme and its degree of activity is an exponential one, as would be expected from the fact of its acting by its surface. Small concentrations are, relatively, more active than larger ones. This is to be accounted for by the fact that the rate of the reaction is determined by the amount adsorbed. It is impossible to assign definite numerical values to the exponents of different reactions, since they vary with the relative concentration of enzyme and substrate. In the middle of the reaction, with the usual amount of enzyme, it is generally just below -2, or thereabouts. This is the "square-root law," which is a rough approximation for a particular stage of the reaction.

There are certain agents which affect the rate of enzymic reactions by a special action on the catalyst. Such are electrolytes, co-enzymes, and "anti-enzymes."

Electrolytes, especially hydrogen and hydroxyl ions, have a powerful effect. Neutral salts also have an influence, as a rule, of a favourable kind.

Some enzymes require the presence of another substance in order to exert their activity. This other substance, known as "co-enzyme," acts in a different manner in different cases. In some it increases the active surface of the enzyme by greater dispersion, in others its mode of action is more specific and as yet obscure.

There is considerable doubt whether true anti-enzymes, whose nature is explained in the text, have any existence. Some of the effects described as being due to them are to be accounted for by changes of hydrogen ion concentration, others to adsorption of the enzyme by a colloid. That of intestinal worms is a peculiar substance, having none of the properties of an antibody in the sense of the theory of immunity.

Contrary to what mass action would predict, it is only in moderate concentrations of substrate that the rate of reaction is proportional to this concentration. Above a certain value, differing according to the case, the velocity of the reaction either remains constant or may even decrease. The cause appears to be of various nature, viscosity, adsorption-saturation of the enzyme, or removal of water. But, where the question has been investigated, the composition of the system in equilibrium is as the law of mass action requires, so that the anomalous effect of increase of concentration only relates to the *rate* of change.

The rate of enzymic reactions is greatly accelerated by rise of temperature. The optimum temperature is merely that at which the increased rate due to the rise is in greatest preponderance over the simultaneous increased rate of destruction of the enzyme.

The importance of regarding reversible or balanced reactions from the dynamic point of view is insisted upon.

The law of mass action shows that, in order to obtain much synthesis, concentration of water must be decreased as far as possible. In the living cell there are probably effective mechanisms for doing this. At the same time, if the synthetic products are continually removed in any way, a small degree of synthesis may result in a considerable amount of products, since the reaction is always going on towards its equilibrium.

There is no evidence for the existence of enzymes which either hydrolyse only or synthesise only. In fact, if enzymes are catalysts, the one agent must do both.

It is possible that an intermediate chemical compound may be formed between the surface of the enzyme and the substrate preliminary to decomposition, but there is no actual evidence that such is the case.

The reacting substances, such as water and substrate in a hydrolytic reaction,

are certainly brought into very intimate contact by adsorption on the surface of the enzyme, and the question is still an open one as to whether this fact, combined with the special nature of the surface itself, is not a sufficient explanation of the increased rate of reaction. The special nature of the surface referred to may be merely physical, but the action of particular enzymes on particular substrates has to be accounted for.

The interaction of electrical forces in the action of neutral salts on adsorption is to be taken into account.

The chemical nature of enzymes is probably very various. There is direct evidence that some are not proteins, and it is doubtful whether any are. Some appear to be complex systems of colloids with inorganic components, or other simple compounds.

There are three stages in heterogeneous reactions—diffusion, adsorption, and chemical reaction. The actual rate of the reaction depends on the slowest member of the series. In colloidal solutions, diffusion and adsorption are rapid, so that the chemical reaction proper is the determining one, a fact which accounts for the high temperature coefficient. But the rate of the chemical change itself is determined by the amount of substrate adsorbed at a given moment, according to the law of mass action.

Some enzymes can be obtained in a stage of formation in which they are inactive, and are then known as "zymogens." These are converted by certain agents into the active enzymes, a change which does not appear to be reversible.

While many enzymes seem to be very "specific," or selective, in that their effect on one particular substrate is very much greater than on any other, it is necessary to be cautious in assuming this as being unconditionally true. Further investigations are needed of the changes in the action of enzymes produced by different conditions. There is also, in many cases, evidence that the same enzyme may act on different substrates at such different rates that it appears to act only on one, unless prolonged observations are made, but the reason why the rate is faster in the one case requires elucidation. Optical isomerism certainly plays a part.

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CHAPTER XI

SECRETION

We have seen in the preceding chapters how important is the function of enzymes in the regulation of the chemical changes of living organisms. Now there is a liquid, one amongst others of the same class, and known as the pancreatic juice, which is formed by the cells of a certain organ and poured into the cavity of the intestine. Its chief properties are due to the variety of enzymes which it contains, although there are other substances present. It may be considered as a typical case of secretion. The cells of the pancreas produce substances which are not present in the blood bathing them and, at the same time, they separate water from the blood in order to carry off these substances in solution. Along with the water we find, as a rule, some other constituents of the blood transferred to the secretion, especially diffusible salts, such as sodium chloride.

There are, however, included under the general name of secretion, the activities of such an organ as the kidney, whose chief function is to separate from the blood certain products of metabolism, such as urea, which would be injurious to the organism unless removed. There are, moreover, the so-called "internal secretions," where substances having special actions on other parts of the organism are formed, but, instead of leaving the cells in which they are produced by a surface in connection with a special channel, the duct of the gland, they are sent in the other direction into the blood current. In such cases, materials supplied by the blood are converted by the organ in question into "chemical messengers" or "hormones," and returned to the blood in this altered form.

It will thus be seen that, under certain aspects, the process of secretion is a part of the general cell metabolism, especially in the case of the internal secretions The manner in which the passage of water is effected in the case of the typical external secretions is a question of much interest, together with the way in which it is regulated. The influences at work causing the production of the specific contents of the secretion will also require our consideration.

In the present state of knowledge, it is impossible to treat the subject from a really general point of view. Perhaps we may look upon the transfer of water from the blood to the secretion as a property common to the majority of cases, so that this phenomenon will be discussed in the first place. It will afterwards be necessary to take special instances, and, as far as possible, our chief attention will be given to those points of most general application.

SECRETION OF WATER

The most obvious hypothesis to make is that the layer of cells forming the membrane intervening between the blood vessels and the lumen of the duct has the properties of a semi-permeable membrane, so that, supposing the pressure in the blood vessels to be higher than the osmotic pressure of the blood, pure water will be forced through. But the osmotic pressure of the blood, as we have seen (page 165), is as high as 6·5 atmospheres, or 5,000 mm. of mercury, whereas 200 mm. of mercury is a high value for the blood pressure. Such a hypothesis is clearly an impossible one. But it is very rarely, if ever, that a secretion consists of pure water, so that the difference of osmotic pressures is less than that given. If the membrane, or one of the membranes, is permeable to the crystalloids but not to the colloids of the blood, such as a gelatine membrane, a very much lower arterial pressure will suffice to filter off a solution containing all the crystalloid components of the blood, in the same concentration as in it. We shall presently see reason to believe that this is the case with the "glomerulus" of the kidney,

where the liquid secreted is blood plasma *minus* its colloids. Certain crystalloids adsorbed on the colloids may be held back by the latter.

Although the secretion of water in general is not a simple process, there are grounds for holding that osmotic phenomena play an important part in it.

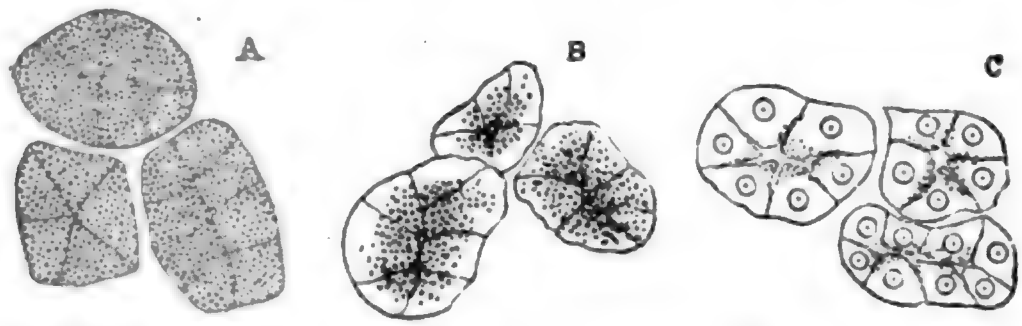

FIG. 88. ALVEOLI OF SEROUS GLAND OF RABBIT.—Fresh, without any addition. All figures from the same gland. The boundaries between the cells are made too obvious in all.

A, At rest.

B, 1·45 hours later, after 3·65 c.c. of saliva had been secreted under the influence of pilocarpine in small doses.

C, Five hours later than *A*, after stimulation of the sympathetic nerve for about two hours, with intervals of rest. 1·6 saliva secreted. The nuclei should not be shown so clearly, although they are unobscured by granules.

(Langley, *Journ. of Physiol.*, **2**, Pl. 7, Figs. 1, 2, and 4.)

We have seen (page 163) how a tube containing a solution of some substance, closed at one end by a membrane impermeable to the solute, and at the other end by a membrane permeable to it, and immersed in water, gives a continuous current of water, or rather solution, issuing from the permeable end, as long as any osmotically active substance is left in the tube. Such a mechanism has been described by Lepeschkin (1906) in the fungus *Pilobolus*, and in the hydathodes of higher plants.

If, therefore, we are justified in assuming that the secreting cells of such organs as the salivary glands or the pancreas are possessed of a membrane on the ends next the blood vessels of such a kind as to be impermeable to some substances produced in the cells, while on the ends next the duct the membrane is permeable to these substances, we can account for a flow of water as long as these osmotically active substances are being formed. They are, of course, carried out with the secretion through the membrane permeable to them.

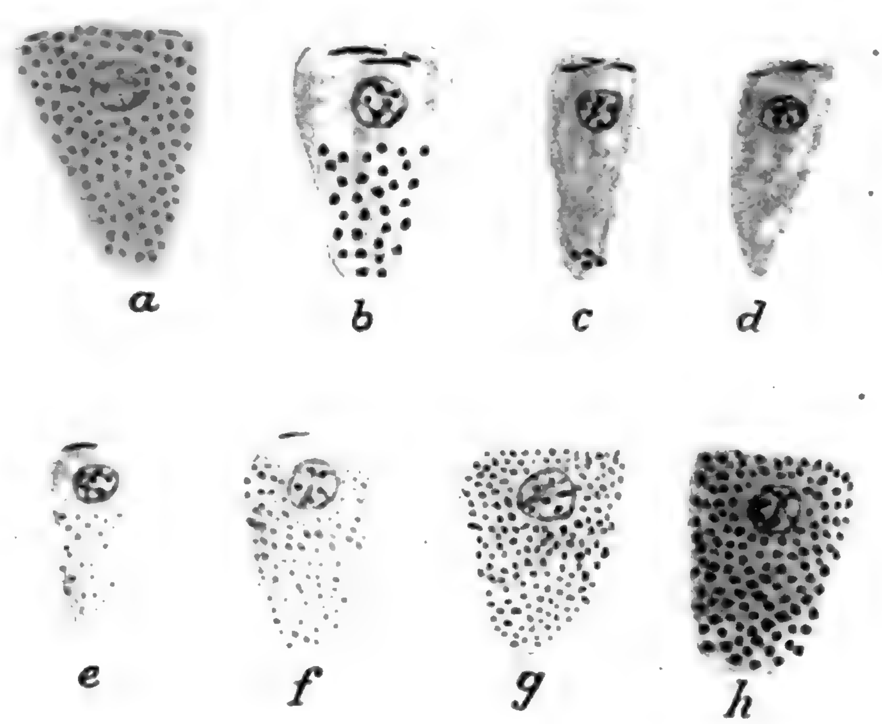

FIG. 89. SEROUS GLAND OF THE HUMAN TONGUE.—Fixed preparation. Diagrams of the series of functional states (*a* to *g*) from the charged resting state through activity to the state of rest again (*h*). According to the work of Zimmermann.

When the secretion has an osmotic pressure higher than that of the blood, it is clear that a mechanism as simple as that described is insufficient, and additional complications, so-called "protoplasmic activities," must intervene, in order to afford the energy necessary to raise the osmotic pressure. Moreover, in any case, except simple filtration, the mechanism in question requires the continuous production in the cells of osmotically active substances. The osmotic pressure of milk, bile,

saliva, and sweat is, in fact, lower than that of the blood, but urine, as it leaves the kidney, has an osmotic pressure considerably higher than that of the blood, owing to increase of concentration as the filtrate from the glomeruli passes along the tubules. Osmotic work must therefore be done by the kidney cells.

The reader will probably note that a mechanism the reverse of that sketched above, namely, cells with their permeable and semipermeable membranes interchanged in position, would account for absorption of water from such cavities as that of the intestine.

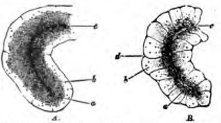

FIG. 90. ALVEOLI OF THE RABBIT'S PANCREAS, LIVING, IN SITU.

a, Inner granular zone.
b, Outer transparent zone, with faint striation in *B*.
c, Lumen of alveolus, obvious in *B*, indistinct in *A*.
d, Indentation at junction of two cells, resulting from decrease in volume.

(Kühne and Lea.)

A fact which points to the intervention of osmotic processes in the secretion of water is the discovery of Ludwig (1851) that the pressure in the duct under which saliva still continues to be secreted is considerably higher than that in the arteries. Hill and Flack (1912) found, with an arterial pressure of 130 mm. of mercury, a pressure of saliva as high as 240 mm. of mercury. This pressure might be brought about either by the production within the cells of osmotically active substances, in conjunction with a membrane impermeable to them, and situated next the blood vessels, or by some process of imbibition, by which swelling of some constituents of the cells takes place. It is more difficult, on the latter view, to see how a continuous secretion could be provided for.

FIG. 91. AN ACINUS OF THE SUBMAXILLARY GLAND OF MAN (EXECUTED).—Fixed and stained by Flemming's method. Obj. 2 mm., Oc. 8.

e, Ergastoplasm.
b, Secretory capillaries.

(Garnier, "Cellules glandulaires séreuses," Nancy, 1899, Fig. 16.)

Changes in the Microscopic Appearance of gland cells might perhaps be expected to throw some light on the question before us, but the interpretation of those phenomena which have been observed is not an easy matter. For details, the reader is referred to the article by Metzner (1907, 1). It must suffice to mention here that it appears to be almost universal that granules ("zymogen" granules) make their appearance in the more or less homogeneous protoplasm of the resting cell, and slowly increase in size. When the gland is excited to secretion, these granules usually undergo a process of solution, and are regarded as the source of the special constituents of the secreted fluid. Sometimes the granules pass directly into the secretion without preliminary solution. It is conceivable that, in the breaking up of these granules, substances of a much smaller molecular weight and higher osmotic pressure than themselves might be formed. During the process of continuous secretion, it is clear that the granules must be renewed, although it is not difficult to obtain, by artificial stimulation of the gland, a nearly complete disappearance of the granules, those that are left being situated next the lumen of the duct. Figs. 88 to 90 show this result in different cases. See also Fig. 92 on page 347 below.

After activity the cells are seen to be diminished in volume. Bunch (1900) showed that, when the submaxillary gland is caused to secrete, there is a rapid decrease in volume of the whole gland, although the simultaneous vascular dilatation in itself produces an increased volume.

In cells which have been fixed, the granules referred to stain with the so-called "acid," that is, electro-negative dyes, such as eosin and acid fuchsin. They behave, therefore, like electro-positive colloids, and in an opposite way to the general mass of the cell protoplasm and the nucleus. There is also frequently to be seen what appears to be a specially differentiated part of the cytoplasm, known as "kinoplasm" or "ergastoplasm," staining deeply with "basic" dyes. This is shown in Fig. 91. According to Laguesse and Debeyre (1912), the dye known as Janus-green brings out the filaments of ergastoplasm in the fresh cell, so that they seem to be present in the living cell, and not to be produced by the fixation process. This dye also stains a little cap of matter on each zymogen granule, which is itself unstained. Some further particulars with regard to morphological changes in gland cells will be found below, in the discussion of the pancreatic secretion.

That there is a *change of permeability* in the secreting cell is indicated by the experiments of Garmus (1912) referred to above (page 140). Under atropine, which paralyses the secretory process, the gland cells are less permeable to dyes than when secreting under the influence of pilocarpine. According to Gildemeister (1913), the cell membrane of the sweat-glands becomes more permeable when activity is brought about by stimulation of the nerves to the glands. This is indicated by the diminution of galvanic polarisation, presumably due to increase of permeability of the membrane to ions, in a way similar to that described above in the case of Congo-red and parchment paper (page 161).

According to Macallum (1911, p. 644), *differences of adsorption*, due to surface tension, play a part in secretory processes. Taking the distribution of potassium as an index to that of the other cell constituents which lower surface tension, he finds, in secretory cells, that there is considerable accumulation of this substance at the cell surface next the lumen. It seems possible that this fact may play a part in the transfer of substances from the body of the cell to the lumen of the duct, although it is difficult to understand how adsorbed substances can play a part in the processes of osmosis or diffusion, since they are held by surface forces. In connection with the remarks made above (page 335) on the possible relation between secretion and absorption, it is interesting to note that, in intestinal cells engaged in absorption, the greater accumulation of potassium is at the end *opposite* to the lumen of the intestine. During absorption of fat, also, it has been noticed that the cells of the intestinal villi show a greater accumulation of fat droplets at their attached ends.

THE GLOMERULUS OF THE KIDNEY

It was pointed out by Tammann (1896) that, if the arterial pressure exceeds the osmotic pressure of the blood colloids, while the membrane of the glomerulus is impermeable to these but permeable to crystalloids, then a solution will be filtered through containing all the latter as in the blood, but devoid of colloids. Tammann obtained incorrect values for the osmotic pressure in question, but Starling (1899), by making use of Martin's gelatine filter, prepared the colloid-free filtrate from serum and compared the osmotic pressure of the original serum against it, thus obtaining the osmotic pressure of the colloids, which amounted to about 30 mm. of mercury. As pointed out above, this was the first definite proof that colloids could have a measurable osmotic pressure. It has long been known that the secretion of urine stops when the arterial pressure falls below 30 to 40 mm. of mercury and Starling brought the two facts into relationship. He also measured the difference between the pressure in the ureter and the arterial pressure, when the former was gradually raised until secretion ceased. At this point the ureter pressure was 92 mm. of mercury when the arterial pressure was 133 mm. of mercury, a difference of 41 mm. of mercury. It will readily be seen that the rate of filtration under a given blood pressure will be increased by reducing the osmotic pressure of the colloids, by dilution, for example. Such a dilution can be produced by the injection of hypertonic solutions, say of glucose, into a vein; the effect is the withdrawal of water from the tissues into the blood.

a state known as "*hydræmia*" or "hydræmic plethora." The same result can be brought about more simply by the injection of a quantity of isotonic saline. In both cases a large rise in the rate of urinary secretion results. It was further shown by Knowlton (1911) that if a colloid, such as gelatine or gum acacia, was added to the saline, so that the osmotic pressure of the colloid added was equal to that of the serum colloids, injection of such solutions caused scarcely any increase of flow. Again, Barcroft and Straub (1910), by the ingenious device of replacing a great part of the blood plasma by Ringer's solution, retaining the blood corpuscles to supply oxygen, were able to obtain a greatly increased rate of secretion without rise of blood pressure.

The experiment is of sufficient interest to be described in more detail. A rabbit with a blood pressure of 95 mm. of mercury was secreting 0·05 c.c. of urine per minute; 22 c.c. of blood were removed, and replaced by 25 c.c. of Ringer's solution. The secretion rose to 0·4 c.c. per minute, with a blood pressure of 52 mm. of mercury. The blood which had been removed was centrifuged, the corpuscles washed, and made up with Ringer's solution to the volume of the blood withdrawn. This was then injected; the blood pressure rose to 84 mm. of mercury, nearly as high as it was originally, while the flow of urine rose to 2·35 c.c. per minute, or nearly fifty times as rapid as the original one, and six times as rapid as that after simple saline injection to replace the volume of the blood taken out. The authors point out the possibility of great variations in the necessary filtration pressure by comparatively small changes in the osmotic pressure of the colloids. Thus, suppose the pressure in the glomerular blood vessels to be 27 mm., and the osmotic pressure of the colloids to be 25 mm., the pressure available for filtration would be 2 mm. Suppose the osmotic pressure of the colloids to be reduced by one-fifth, so that it becomes 20 mm., then the filtration pressure becomes 7 mm., or 3·5 times as great as before.

From these various experiments it is clear that the filtration hypothesis is capable of accounting for the production of a urine which is equivalent to the blood plasma minus its colloids.

If the glomerular process is merely a filtration, it is clear that what work is required for it is afforded by the blood pressure, in other words by the heart, so that the cells of the glomeruli take no part in the performance of work. Barcroft and Straub (1910) have made observations on the oxygen consumption of the kidney which confirm this point of view. No increase in the oxygen consumed occurred when the increase of secretion was brought about merely by dilution of the blood. We shall see presently that where work has to be done, increased oxygen is consumed in order to give energy by oxidation.

A subsidiary point of interest in this connection, which also gives support to the filtration theory, is that, if there were some special function of the cells of the glomerulus in the nature of selective secretion, the kidney would not continue to turn out an important salt, such as sodium chloride, when the organism has been deprived of it in the food. Cohnheim's experiments (1912, p. 80) show that the contrary is the case. On the filtration hypothesis, sodium chloride must appear in the liquid leaving the glomerulus as long as there is any of it free in the blood. We shall see, however, that it may be reabsorbed to a considerable extent in the tubules.

A certain difficulty must not be overlooked. The urine of the frog, and of water animals in general, as it leaves the kidney, is of a *lower* osmotic pressure than that of the serum minus its colloids. After copious drinking of watery fluids, this may also happen in man. It is to be remembered that we have no direct knowledge of the composition of the solution as it leaves the glomerulus in such cases, and it is known that the tubules are able to absorb valuable stuffs from the glomerular filtrate as it passes over their cells; although, when the flow is very rapid, there does not seem to be much time given for the process. It has been held that the tubules secrete water, but there does not seem to be any evidence for this. The functions of the tubules will be discussed later, and we shall see that, on the view for which Cushny (1917, p. 143) brings powerful evidence, the tubules absorb a fluid which is practically identical with Locke's Ringer solution, so that if the glomerular filtrate is more dilute than normal it becomes still further diluted by the removal of solution which is more concentrated than itself.

Brodie (1914) calculates that the pressure required to drive urine down along the tubules at the rate of diuresis is practically identical with the blood pressure in the glomeruli, so that only one or two millimetres at most would be available for a filtration pressure. The difficulty of accepting the calculation rests on the fact that the fourth power of the radius of the

capillary tube enters into Poiseuille's formula, which was used, so that a slight difference in this value would make a large one in the result, and Brodie's measurements are made on a hardened kidney. Apart from this, it is very difficult to believe that a pressure of 83 mm. of mercury could exist in the glomerulus end of the tubule without causing great dilatation. If this distension were prevented by the resistance of the capsule of the kidney, it would surely result in obstruction of the capillaries and veins. There is no evidence of a strong muscular coat to the tubules, such as there is in the arterioles of the glomerulus. The conclusion drawn by Brodie from his calculation is that the glomerulus must be an actively secreting organ for water, and that the water is driven on in some way by the aid of blood pressure. But if we grant that the cells act as secreting organs, like those of the salivary glands, why is it necessary to assume any further driving pressure?

THE WORK DONE IN SECRETION AND THE CONSUMPTION OF OXYGEN

Although the glomerular filtrate of the kidney has no higher osmotic pressure than that of the blood plasma, it is well known that the urine, as it leaves the kidney, has a much higher molar concentration. Work must therefore be done in the total process. This work can be calculated in the way indicated in a previous chapter (page 33), and will be described presently.

Chemical Work.—In the production of the special constituents of any secretion, chemical work must be done by the gland cells. Moreover, the source of the energy required for their osmotic work has also its origin in chemical reactions in the cell systems. We have no direct way of measuring this work, but the amount of oxygen consumed, or the carbon dioxide given off, by the gland in different states of activity, gives us valuable indirect information. The energy at the disposal of the cells is derived from the oxidation of substances of high chemical potential to substances of low chemical potential, such as carbon dioxide and water. In the process, a part of the free energy is degraded to heat and carried off by the blood current, so that it is impossible in practice, at all events as yet, to obtain an absolute measurement of the amount of work required to form a given amount of secretion.

Osmotic Work.—It is advisable to give some further details of the method of calculating this, in addition to those already given in a general form when discussing the formula for the isothermal compression of a gas.

We must recollect that we cannot look upon the urine as merely a concentrated glomerular filtrate, as was done by Dreser (1892) in the first approximate calculation of the renal work. The relative proportion of the constituents is not the same. For example, the ratio of sodium chloride to urea in the blood (or glomerular filtrate) is about 10 to 1, whereas in the urine it is reversed, and becomes 1 to 2. Thus, while the osmotic pressure of the sodium chloride has only to be raised from that of a 0·18 molar solution to that of a 0·36 molar one, or about doubled, that of the urea has to be raised from a 0·01 molar strength to that of a 0·4 molar strength, or increased forty times.

It will be best, however, to obtain first the work done, as Dreser did, on the supposition that we are dealing only with an increase of concentration, leaving for the present the fact that the various constituents are unequally affected.

At the outset it is well to call attention to the fact that the results obtained by such methods of calculation are valid, whatever be the exact mechanism by which the process is brought about in the organism.

On page 33 we saw that the expression which gives us the work done in compressing a gas isothermally from a volume v_1 to a volume v_2 is—

$$A = RT \log_e \frac{v_1}{v_2},$$

or

$$= 2{\cdot}303.RT \log_{10} \frac{v_1}{v_2}.$$

R, of course, can be expressed in any convenient units, and is—

0·0821 for litre atmospheres,
1·991 for gram calories,
or 0·848 for kilogram metres;

according to the units in which the work is to be expressed.

and T at 37° C. is 310°, so that—

$$A = \left.\begin{array}{r} 58{\cdot}61 \text{ litre atmospheres,} \\ 1421{\cdot}4 \text{ gram calories,} \\ \text{or } \quad 605{\cdot}5 \text{ kilogram metres} \end{array}\right\} \times \log \frac{v_1}{v_2}.$$

The same expression, as we saw before, applies to the alteration of the concentration of a solution when produced in any way. Of course, when electrolytes are concerned, changes in electrolytic dissociation must be taken into account.

Incidentally, it may be remarked that, as regards calculations involving energy factors, living organisms have the advantage of their reactions being carried on in a practically isothermal system, so that the formulæ are comparatively simple. Any change of temperature is so small as to have only a minimal effect on the results of the calculation.

To proceed, the total osmotic concentration of the blood is about 0·3 molar, and from this, under ordinary conditions, the kidneys produce a urine which is about molar, that is, the osmotic pressure is increased rather more than threefold.

Instead of volumes in our formula, it is convenient to take concentrations, which are the reciprocals of the volumes in which 1 gram-molecule is dissolved. Further, since the osmotic pressure (π) and the depression of the freezing point ($\triangle$) are also in direct relation to one another, we can take, in place of $\frac{v_1}{v_2}$, either $\frac{c_2}{c_1}$, $\frac{\pi_2}{\pi_1}$, or $\frac{\triangle_2}{\triangle_1}$.

Strictly speaking, the use of the last expression is only permissible at the temperature of the freezing points in question, since electrolytic dissociation may not be the same. But the experimental error of the freezing point measurements exceeds the very small errors possible on account of differences of dissociation.

Accordingly, the minimal work which the kidney must do in order to produce, from a glomerular filtrate of $\triangle_1$, a urine of $\triangle_2$, in an amount which contains 1 gram-molecule at 37° is—

$$A = 58{\cdot}61 \log \frac{\Delta_2}{\Delta_1} \text{ litre atmospheres} = 1421{\cdot}4 \log \frac{\Delta_2}{\Delta_1} \text{ gram calories}$$

$$= 605{\cdot}5 \log \frac{\Delta_2}{\Delta_1} \text{ metre kilograms.}$$

If n mols. are compressed instead of one, the work is increased n-fold. The molar concentration, in practice, can be best obtained from the depression of the freezing point, to which it is related, as we have seen (page 155), and in the following way:—

$$n = \frac{\Delta v}{1{\cdot}85}$$

which gives the number of mols in v litres of solution.

The depression of the freezing point of urine ($\triangle_2$) is between 1°·5 and 2°, so that, for simplicity of calculation, we may take it as 1°·85, and there are, in man, about 1·5 litres produced per day, hence:—

$$n = \frac{1{\cdot}85 \times 1{\cdot}5}{1{\cdot}85} = 1{\cdot}5,$$

Δ_1 (that of blood) is 0·56.

Inserting these values in the above equation, we have—

$A = 1{\cdot}5 \times 58{\cdot}61 \times \log \frac{1{\cdot}85}{0{\cdot}56} = 45{\cdot}6$ litre atmospheres, as the daily osmotic work of the kidneys.

But, as Dreser points out, we must remember that to produce 1·5 litres of urine of $\Delta = 1°{\cdot}85$ from blood of $\Delta = 0°{\cdot}56$,

$$1{\cdot}5 \times \frac{1{\cdot}85}{0{\cdot}56} = 4{\cdot}955 \text{ litres of blood are required.}$$

In the calculation, the difference between this quantity and that of the urine secreted, namely, $4{\cdot}955 - 1{\cdot}5 = 3{\cdot}455$ litres, has been reckoned as pure water. In

point of fact, it is kept in the blood and at a Δ of 0°·56. We have thus made the work of the kidneys too great by the amount required to raise 3·455 litres to the osmotic pressure corresponding to a Δ of 0°·56. A Δ of 1°·85, as we saw (page 155), is equivalent to the osmotic pressure of a molar solution, that is 22·4 atmospheres; therefore, 0°·56 means an osmotic pressure of $22{\cdot}4 \times \frac{0{\cdot}56}{1{\cdot}85} = 6{\cdot}8$ atmospheres at the freezing point, or 7·7 atmospheres at 37°. We have to subtract, then, $3{\cdot}455 \times 7{\cdot}7 = 26{\cdot}6$ litre atmospheres, from our first value of 45·6, leaving 19 litre atmospheres as the correct value, on our simple assumption of mere total concentration.

But, as already remarked, this is not all. We must take account of the relative concentrations of the different constituents of the urine, since they are by no means equally compressed. The urine is not merely a glomerular filtrate boiled down, as it were. This question is treated in the paper by von Rhorer (1905), to which the reader is referred for more details than can be given here. It will be clear that a completely accurate measurement of the total work done could only be obtained by taking each constituent of the urine for itself. As an illustration of the method, we may take the two chief constituents of the urine, sodium chloride and urea, as is done by von Rhorer (pp. 388-390), and, indeed, the osmotic concentration of the other constituents is comparatively small, so that our result will not be far wrong.

Instead of the complex glomerular filtrate, we imagine, in the first place, a pure solution of sodium chloride of the same concentration as that in which it exists in the blood, that is 0·18 molar, inclusive of ions. We have to concentrate this solution to that of the sodium chloride in urine, that is, to 0·36 molar. It will be instructive to treat the problem in the way done by van't Hoff, described in one form on page 157 above. We imagine a cylinder closed at the end, and containing a piston impermeable to sodium chloride, but permeable to all the other solutes of the glomerular filtrate and to water. We compress the filtrate until the concentration of the sodium chloride below the piston is raised to 0·36 molar. In the kidney the concentration is only raised from 0·18 to 0·36, while in our imaginary model no sodium chloride passes through the piston, but water does, so that the original concentration of 0·18 molar above the piston is lowered; we must therefore add continuously sodium chloride to the solution above the piston in order to maintain its concentration constant at 0·18 molar. We keep thus the osmotic pressure above the piston unaltered at p_0, while below it the pressure during the operation is a variable one, p, and is raised gradually from p_0 to $2p_0$ (0·18 to 0·36). The work done consists, then, in raising the pressure of a volume of solution by a series of infinitesimal steps from p_0 to a higher one, through the variable pressure differences of $p - p_0$. That is:—

$$dA = (p - p_0)dv.$$

The integral of this expression consists of two members—

$$A = \int_{v'}^{v} p dv - p_0 \int_{v'}^{v} dv$$

where v is the initial volume and v' the final one, the actual process being performed by the diminution of the volume from v to v'. Since p_0 is kept constant, it is outside the sign of integration, instead of in a place similar to that of p.

The first member we know already (page 33) as

$$RT \log_e \frac{v}{v'}.$$

The second is simply:— $-p_0(v - v')$.

Instead of $\frac{v}{v'}$, we can put $\frac{c'}{c}$ (concentrations instead of dilutions), and since $vc = v'c' = n$ (c being the number of mols. dissolved in v),

$$v' = \frac{n}{c'}, \text{ and } v = \frac{n}{c}.$$

Also, since $p_0 v = RT$, $p_0 = \frac{RT}{v}$ or $= cRT$.

Putting these values in the integral, we have

$$A = n\mathrm{RT}\ 2{\cdot}3 \log \frac{c'}{c} - c\mathrm{RT}n\left(\frac{1}{c} - \frac{1}{c'}\right)$$

$$= n\mathrm{RT}\left[2{\cdot}3 \log \frac{c'}{c} - c\left(\frac{c'-c}{cc'}\right)\right]$$

$$= n\mathrm{RT}\left[2{\cdot}3 \log \frac{c'}{c} - \frac{c'-c}{c'}\right]$$

In our particular case, $n = 0{\cdot}36$, $c = 0{\cdot}18$, $c' = 0{\cdot}36$, and $\mathrm{RT} = 262{\cdot}9$ kg. metres at 37°, so that

$$A = 0{\cdot}36 \times 262{\cdot}9 \left(2{\cdot}3 \log \frac{0{\cdot}36}{0{\cdot}18} - \frac{0{\cdot}36 - 0{\cdot}18}{0{\cdot}36}\right) = 18{\cdot}28 \text{ kg. metres.}$$

We now, in imagination, repeat the operation on this same solution, using a piston which is impermeable to urea, permeable to water and sodium chloride with the other solutes. The compression has to raise c of 0·01 to c' of 0·4; n is 0·4, and therefore A is

$$= 0{\cdot}4 \times 262{\cdot}9 \left(2{\cdot}3 \log \frac{0{\cdot}4}{0{\cdot}01} - \frac{0{\cdot}4 - 0{\cdot}01}{0{\cdot}4}\right) = 290 \text{ kg. metres.}$$

The total work is therefore

$$18{\cdot}28 + 290 = 308{\cdot}28 \text{ kg. metres.}$$

By the simple process of calculation of total concentration by which a glomerular filtrate of initial concentration of $0{\cdot}18 + 0{\cdot}01 = 0{\cdot}19$ molar ($= c$) is raised to one of $0{\cdot}36 + 0{\cdot}40 = 0{\cdot}76$ (c') by aid of a piston impermeable to urea and sodium chloride, we have, since $n = 0{\cdot}76$—

$$A = 0{\cdot}76 \times 262{\cdot}9 \left(2{\cdot}3 \log \frac{0{\cdot}76}{0{\cdot}19} - \frac{0{\cdot}76 - 0{\cdot}19}{0{\cdot}76}\right) = 127{\cdot}2 \text{ kg. metres.}$$

Thus, when we take account of the different partial pressures of urea and sodium chloride, we obtain 2·5 times as great an expenditure of work.

It may be pointed out that the work calculated in this manner is simply that necessary to effect the change of molar concentrations, and is independent of any particular process by which it is effected. The actual work done by the cells depends on the efficiency, in the engineer's sense, of the machinery by which the energy is afforded. The method can therefore equally well be used to find the osmotic work necessary to secrete a liquid more dilute than blood, as is done by von Rhorer (pp. 383, 384).

Since n in the above formula is identical with c', owing to the concentration being expressed in molar values, we may write :—

$$\mathrm{RT}\left[c' \log \frac{c'}{c} - cc'\left(\frac{c'-c}{cc'}\right)\right]$$

that is:—$\mathrm{RT}[c' \log \frac{c'}{c} + c - c']$ as given by Barcroft (1914, p. 94).

Or, if all the constituents are taken account of :—$\mathrm{RT}[\Sigma\left(c' \log \frac{c'}{c}\right) + \Sigma c - \Sigma c']$

as given by Cushny (1917, p. 33).

Alkaline and Acid Secretions.—This process may be looked upon, from the point of view of the present section, as the change of concentration of hydroxyl or hydrogen ions of the blood into that of the secretion, or as one of the osmotic partial phenomena, as dealt with above in the case of urine. Von Liebermann (1911, p. 34) points out how, in the case of the alkaline pancreatic juice, diminution of the OH' ion concentration of the blood by intravenous injection of lactic acid causes a reduction in the rate of flow of the secretion under a constant stimulus. This might be explained as due to the greater work necessary to raise the OH' ion concentration in the juice to the same height from a lower level; but there may, no doubt, be other factors in addition. The mechanism of secretion of acid and alkali will be referred to again later (page 359).

Mention has frequently been made of the use of intravenous injections for various purposes, so that it may interest the reader to learn that, according to Sprat's "History of the Royal Society" (1722, p. 317), it was Christopher Wren who was, as the author puts it, "the first Author of the Noble Anatomical Experiment of Injecting Liquors into the Veins of Animals. An Experiment now vulgarly known; but long since exhibited to the Meetings at Oxford, and thence carried by some Germans, and published abroad. By this operation divers Creatures were immediately purg'd, vomited, intoxicated, kill'd or reviv'd, according to the quality of the Liquor injected. Hence arose many new Experiments, and chiefly that of Transfusing Blood, which the Society has prosecuted in sundry instances, that will probably end in extraordinary Success." (See also Bayliss, 1918, pp. 151-152.)

Secreting glands also require *energy* for the production of the *chemical* constituents of their secretions, whenever these substances are not already present in the blood.

When we come into possession of more knowledge of the chemical changes involved, it is possible that we may, by the application of Nernst's new thermodynamic theorem (page 30 above), be able to calculate the energy changes involved. For the present we must be content with indirect measurements by determining the difference between the *oxygen consumption* of the resting and the active organ. This knowledge we owe chiefly to the work of Barcroft and his co-workers. The measurement is made by determining the oxygen content of the blood supplied to the gland, that is, the ordinary arterial blood, and the oxygen content of that leaving it by the vein, together with the amount of blood passing in a given time. It is in the accuracy of the last estimation that the chief difficulty lies, since the rate of flow increases in activity. The resting submaxillary gland of the cat consumes about 0·02 c.c. of oxygen per gram per minute. When excited to secretion, the consumption may rise to as much as 1·9 in the same units (Barcroft and Piper, 1912, p. 362), that is, more than five times as much. By taking the difference between the oxygen consumption of the resting and that of the active gland, the same observers have calculated the oxygen necessary to form 0·30 c.c. of saliva to be 0·18 c.c. What this means in terms of energy naturally depends on what chemical substance is oxidised. Taking it as glucose, it would imply the use of 0·17 g., since 180 g. of glucose require 192 g. of oxygen for complete combustion. A small part only of the energy is required for osmotic work, on account of the small volume of the saliva secreted.

A very important result as regards the mechanism of the process was obtained in the course of the experiments of Barcroft and Piper. When the time course of the oxygen consumption was determined in relation to that of the flow of saliva, it was found that the maximal rate of the former occurred considerably later than that of the latter, and that the increased consumption might last for as long as seven minutes after the formation of saliva had ceased. The length of this period of increased consumption of oxygen was found to depend on the degree of activity of the gland previously, and also on the functional capacity of the organ. We shall meet with a similar state of affairs in the case of voluntary muscle. It seems to imply that the chemical energy derived from oxidation is not used directly in the process of secretion, but that potential energy is stored in some physico-chemical system, from which it is given out for use in the actual process itself.

It might perhaps be thought, by adherents of the "biogen" theory, that the oxygen itself is taken up in combination in an explosive-like giant molecule, analogous to potassium chlorate, for example. It seems possible that this view might be tested by simultaneous determination of the carbon dioxide given out together with the oxygen consumption. If the two were found to correspond, it would indicate that an oxidation process was giving energy to another independent chemical or physical reaction. Want of parallelism between the oxygen and carbon dioxide would not decide the question in either way. We shall find later, however, that there is no evidence for the existence of "intramolecular" oxygen in the sense of the biogen hypothesis, and we have already seen reason for doubting the correctness of this point of view.

It may be called to mind that Chauveau and Kaufmann (1886) found a diminution of glucose in the blood after it had passed through the active salivary gland of the horse. This was also found to be the case by Asher and Karaulov (1910), so far as the period immediately succeeding the flow of saliva is concerned. The fact suggests the possibility that the energy required to form the system of high potential energy, which afterwards breaks down in the process of secretion, may be derived from the oxidation of glucose.

It is remarkable that the latter investigators found an *increase* of glucose in the venous blood of the gland during the process of secretion itself; they hold that it may be a constituent of some substance which breaks down in secretory activity. This does not seem a very probable thing to happen, as it would not be capable of affording much energy. It is also difficult to understand why the glucose does not appear in the saliva. It seems, moreover, that the concentration of the venous blood, due to loss of water into saliva and lymph, has not been sufficiently taken into account in the experiments referred to. For example, in experiment 1 (p. 40 of the paper), during the two minutes necessary for collection of the 10·57 c.c. of venous blood for analysis, 4 c.c. of saliva were secreted; adding this to the blood makes 14·57 c.c. and the percentage of glucose must be diminished in the same ratio, which makes it 0·148 per cent., a value practically equal to that in the arterial blood supplied to the gland, and no account has been taken of the lymph, which would make the value in the venous blood *less* than that in the arterial blood. From the results of Barcroft and Piper, it is not to be expected that there would be any very considerable consumption of glucose during the first period of the activity of the gland.

In Barcroft and Brodie's work (1905, p. 65) on the gaseous metabolism of the active kidney, it was found that, taking all the experiments together, the output of carbon dioxide was equivalent to that of the oxygen taken in. That is, the respiratory quotient (see above, page 279) is practically unity, as it would be from the oxidation of carbohydrate only. So far as it goes, this result suggests that the substance oxidised is of carbohydrate nature and that it is completely oxidised and its energy used for some process in connection with secretion. The oxygen, moreover, could not be combined up in an intramolecular form in an "explosible" substance, since, if this were the case, the respiratory quotient $\left(\frac{CO_2}{O_2}\right)$ would be greater than unity during the period of formation of this substance and less than unity during its breaking up.

The great sensitiveness of the salivary glands to slight diminution of oxygen supply, as found by Heidenhain (1868, pp. 88-101), by Jonescu (1909, p. 68), and by Liebermann (1911, p. 26), shows that the process of formation of the secretion itself requires free oxygen in addition to the stored energy just referred to. Ludwig, however (1851), obtained a slight secretion after the circulation had nearly ceased, so that a current of blood is not absolutely necessary. This consumption of oxygen during actual secretory activity suggests that the system of high potential energy, formed previously, does not contain in itself the oxygen necessary for its combustion, but chiefly consists of an oxidisable substance capable of affording energy when supplied with oxygen.

This immediate dependence on oxygen is shown still more strikingly by the higher nerve centres and makes it probable that, for proper functional work, oxygen must be supplied, not only in a certain amount, but at a tension not far below that in which it is present in the atmosphere and in arterial blood. An organ may suffer from want of oxygen even when the venous blood coming from it still contains oxygen, so that oxygen has passed the cells unused.

Formation of Heat.—It is scarcely to be supposed that the efficiency of the gland machinery is so high that no free chemical energy is degraded to heat in the secretory process. It was, in fact, found by Ludwig and Spiess (1857) that the temperature of the saliva coming from the submaxillary gland was higher than that of the blood in the carotid artery, but Bayliss and Hill (1894, 1) were unable to detect any difference in this sense, if care was taken to obtain the actual temperature of the blood in the flowing stream. Ludwig, himself, in a letter to Prof. Schäfer, appears to have been prepared to admit these negative results. Of course, the fact merely shows that, if heat was produced, the blood current was sufficiently rapid to carry it away as fast as it was formed, as is very probable from considerations of the actual amount of the combustion going on. It should be stated also, however, that we were unable to detect any formation of heat in the excised salivary glands of the grass snake, although a very delicate method was used (p. 352 of the paper quoted).

MODES OF EXCITATION

There are two different ways in which glands can be made to secrete. The one is by the agency of chemical substances contained in the blood with which they

are supplied; the other is by stimulation of nerve fibres which terminate in the secretory cells. It may, of course, ultimately be found that, in the actual cell system itself, the processes are identical in the two cases, so that the nerve may act by production of the same chemical substance which excites directly, or the chemical excitant may act on the same terminal mechanism as the excitatory process in the nerve fibre does.

It has long been known that certain *drugs*, of which pilocarpine is the most familiar, are capable of causing practically all glands to enter into activity. The fact that glands, such as the mucous glands of the air passages, which do not appear to be supplied with nerves, are excited seems to indicate that this effect is produced independently of nerve supply. On the other hand, adrenaline causes a powerful secretion of the submaxillary gland of the cat and we know that the action of this substance is exerted on the endings of the sympathetic nerves, wherever they are found. It appears, then, that a chemical substance may excite the cells of glands either directly, or through the medium of the nerve terminations on them. In the former case, it is probable that the drug may act on some definite part of the cell system, the "receptive substance" of Langley (1906). After administration of atropine, pilocarpine is ineffective in producing secretion, nor can it be produced by exciting the nerves of such glands as are supplied with them. Pilocarpine seems to be an abnormal excitant for gland cells, since its action is very violent and profound morphological changes are caused in the cells. In this respect it differs from a normal chemical excitant, such as *secretin* for the pancreas, the mechanism of which we will now consider. It was shown by Pavlov and his fellow-workers (1901, p. 132 of the English translation) that the presence of various substances in the duodenum, especially acids, causes pancreatic juice to be poured in. This excitation of the pancreas was looked upon as a reflex through the nervous system until Bayliss and Starling (1902, 1), in investigating the local nervous reflexes connected with the alimentary canal, found that it was still produced by acid in the duodenum after all accessible nervous communications had been divided. This fact suggested that some chemical mechanism was at work, set going by the acid. The injection of acid into the blood current has no effect, as would be expected, so that some substance must be produced by the action of the acid on the mucous membrane of the intestine, which substance then diffuses into the blood and, arriving at the pancreas, excites it to action. The next step was to scrape off the mucous membrane and rub it up with sand and dilute hydrochloric acid. After neutralisation and filtration, this extract was injected into a vein and we were naturally delighted to find that a copious flow of pancreatic juice was the result. It may be pointed out that it is quite immaterial whether the whole of the nerves were actually cut in the previous part of the experiment, since it was the belief that they were cut that led to the search for a chemical mechanism.

Further details as to the properties of this "secretin," as we called it, being unable to think of a better name, will be found in Chapter XXIV. The name itself has now come into general use and, whatever objection may be made to it, it must be admitted that it has the advantage of making no assertion as to the chemical nature of the substance, as to which we have little positive knowledge.

The juice formed under the action of secretin appears to be identical with that formed during natural digestion; perhaps it may be rather more dilute; it contains trypsin in the inactive, zymogen form, amylase and lipase, together with alkaline salts. The pancreas can be caused to secrete continuously for many hours by repeated doses and, although in the later stages a somewhat more dilute juice may be obtained, it is a matter of considerable difficulty to induce signs of fatigue in the cells, so far as microscopic observation can detect them.

Atropine has no effect on the action of secretin, contrary to its action on secretion produced by stimulation of nerves. Secretin must act, therefore, on the cells directly, or, at all events, on some part of the cells beyond nerve terminations.

How far this natural form of chemical stimulation of glands applies in general remains as yet uncertain. It is comparatively unimportant in the case of the salivary and sweat glands, but the work of Pavlov (1901, Lecture VII.) and of Edkins (1906) shows that the *gastric juice* is partly produced by the agency of

a chemical substance produced in the stomach itself by certain constituents of the food, and that this substance acts through the intermediation of the blood current (see page 372 below), although the gastric glands are also powerfully excited by fibres in the vagus nerve.

Secretion of Bile is produced by the same acid extract of duodenum which excites the pancreas, but whether the same "secretin" is at work we cannot state. The liver can also be excited to secretion by injection into the blood of bile-salts; in such a case, the concentration in the blood of the constituents of the secretion plays a part in determining the activity of the cells in transferring them from the blood to the duct. The blood supply of the liver (Heidenhain, 1880, pp. 259-268), both in respect of rate of flow and of pressure, affects the rate of secretion to a large extent.

The *Succus Entericus* is secreted in a particular section of the small intestine when trypsin is present in a part preceding it in the normal direction of the passage of food. The work of Pavlov (1901, p. 161 of the English edition) tends to show that this is a chemical mechanism.

The *Nervous Mechanism of Secretion*.—The majority of glands, including those with internal secretion, are supplied with nerves by which they can be excited to action by reflexes from the central nervous system. Most of our knowledge is derived from the study of the salivary glands, owing to the comparative ease with which experimental work can be conducted on these organs. We will consider, in the first place, the special case of the submaxillary gland of the dog and afterwards apply the results to other glands. This submaxillary gland is supplied by two sets of nerve fibres, both of which play a part in the secretory mechanism. The first set, contained in the chorda tympani nerve, arise from the brain in the small-fibred portion of the facial nerve, corresponding to the intermediate nerve of Wrisberg in man, which leaves the mid-brain between the facial and auditory nerves (see Gaskell, 1889, p. 172).

These fibres may be called the cerebral supply; the other set comes from the sympathetic system, a special outflow of nerves to the viscera, blood vessels, and similar structures; to this system of nerves attention will be directed in a later chapter.

It is found that excitation of the chorda tympani nerve produces a copious watery secretion, while that of the sympathetic nerve produces a small quantity of a very thick saliva. Heidenhain (1868, p. 113) propounded the view that there are two different kinds of fibres concerned, one set with the secretion of water, together with the diffusible salts present in the blood, and the other set with the formation of the specific solid constituents of the secretion. On this ground he called the former "secretory," the latter "trophic," using this latter word in a rather special sense (1868, pp. 101-104, and 1880, p. 51). The two kinds of fibres are supposed to be present in both nerves, but in different relative amount, which varies according to the kind of animal. In the cat, for instance, Langley (1878) showed that the chorda and sympathetic nerves both give very much the same kind of saliva. The sympathetic nerve contains fibres which cause great constriction of the arterioles of the gland, while the chorda contains fibres which dilate them, so that it has been held (see Langley, 1898, p. 529) that the restricted blood supply is responsible for the relatively concentrated saliva produced by the "trophic" nerve fibres, and that there is no necessity to assume the existence of two kinds of nerve fibres presiding over secretion. The question seems, however, to be definitely decided in the latter sense by the experiments of Babkin (1913), who investigated the properties of the saliva secreted reflexly by placing in the mouth, in the one case, meat powder, in another case, hydrochloric acid. It was found that the blood flow through the gland was equally accelerated by both, but, while the content in inorganic salts was identical, the organic constituents of the saliva secreted under the stimulus of meat were in four to five times as great an amount as in that formed under hydrochloric acid. Since the removal of the superior cervical ganglion, by which the influence of the sympathetic fibres is removed, had no effect on the result, it is necessary to assume that the chorda tympani

nerve also contains "trophic fibres." There was no evidence that vaso-constrictor fibres were excited in either case. Expressed on Heidenhain's view, we may say that acid excites the "secretory" and vaso-dilator fibres, while the "trophic" fibres are very little affected. Meat excites both the secretory and the trophic fibres of both nerves, in addition to the vaso-dilator fibres of the chorda.

Langley (1916) thinks it possible that different parts of the centre may be excited by the different afferent impulses and that cells in the gland secreting different amounts of solid may be excited.

Should it be true that there are two different kinds of fibres to the salivary glands, it seems probable that, whenever we have a liquid secretion containing constituents foreign to the blood, the secretion of these substances is under the control of special nerve fibres; this statement, of course, only refers to those cases where the process is effected by nervous, not by chemical, agency.

The Pancreas.—The chemical mechanism in this case appears to be so adequate and appropriate that its discoverers were inclined to doubt the existence of a nervous mechanism, although we were careful not to deny it (Bayliss and Starling, 1902, p. 343). At that time, the experimental evidence did not exclude the possibility of explanation on the lines of a chemical mechanism, but Pavlov has since brought forward evidence which amounts to a satisfactory proof that the vagus nerve contains fibres that cause the production of pancreatic juice, although there are several peculiar facts in connection with the phenomenon. G. W. Anrep has demonstrated in England the method of experiment, and there is no doubt that secretion can be obtained by exciting the vagus under certain conditions, which have to be pretty closely adhered to. Some reflex inhibitory influence is exercised by the operative procedures, so that it is necessary to divide the spinal cord at the foramen magnum; the secretion does not appear until after several successive periods of stimulation of the vagus nerve and, when it appears, it is much less copious than after secretin, and contains active trypsin. This last fact presents some difficulty in regarding the vagus effect as a normal mode of production of the juice, since Delezenne and Frouin (1902 and 1903) have shown that the juice which appears copiously from a permanent pancreatic fistula, when food is being digested, both in the dog and in the ox, is inactive until acted upon by enterokinase. The action of the vagus is paralysed by atropine, like other gland nerves. From the very concentrated character of the juice it would seem that the vagus contains chiefly "trophic" fibres. The question of inhibitory nerves to glands will be discussed later. The paper by Bylina (1912) on the two kinds of mechanism, chemical and nervous, should be consulted.

The view that there are distinct "trophic" fibres in gland nerves receives further support from the changes in microscopic appearances of the gland cells. Excitation of the sympathetic produces considerably more signs of fatigue in the submaxillary gland cells than that of the chorda does. Babkin, Rubashkin, and Savich (1909) have described similar facts with regard to the pancreas. As already stated, by the action of secretin it is difficult to produce signs of fatigue in the cells, while stimulation of the vagus nerve results in marked changes. According to the observers named, the process of secretion in the case of the chemical excitant is as follows:—Water flows through the cell in quantity, and one sees in the cells what look like channels of fluid (see their Fig. 23). This current carries out the zymogen granules into the ducts, where they can sometimes be seen as granules, but they soon become dissolved. It is found, on staining with eosin and orange, that the secretion in the ducts takes the same red colour as the granules inside the cells, and appears to be of the same chemical nature. We know that the trypsin in it is still in the zymogen stage. No cell constituents staining with orange are to be found.

After nerve stimulation, which gives only a small quantity of a thick juice, we have a different picture. The granules inside the cells undergo a transformation; they gradually lose the property of staining with eosin or iron hæmatoxylin and become stainable with orange, sometimes forming large "vacuoles" before passing into the duct. The secretion itself in the ducts stains with orange, not with eosin (see Figs. 16 and 18 of the paper). As we saw, it contains active trypsin. Little or nothing is to be seen of the intracellular channels of the more watery secretion

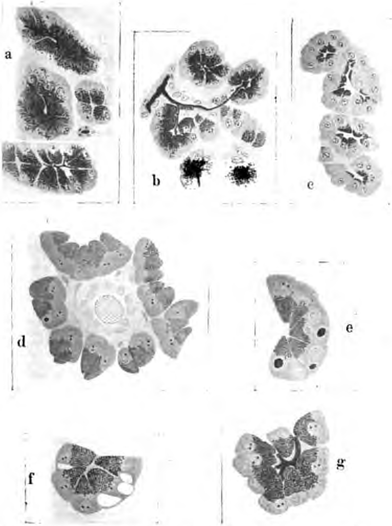

FIG. 92. STAGES OF ACTIVITY OF THE PANCREAS UNDER THE ACTION OF SECRETIN, AND OF STIMULATION OF THE VAGUS NERVE.

a, *b*, *c*, Stained with iron hæmatoxylin.
  a, From fasting dog.
  b, After action of secretin (acid in duodenum).
    62·8 c.c. of juice secreted in four hours and ten minutes.
    Comparatively slight disappearance of granules and little structural change.
  c, After vagus stimulation.
    1 c.c. of juice in the first fifty-three minutes; 5·1 c.c. in the next hour and a half; 6·4 c.c. in the last forty-one minutes.
    Great disappearance of granules.
d, *e*, *f*, *g*, Stained with eosin-orange-toluidine blue by Dominici's method. The parts stained with eosin are shown black; those stained orange (drops of secretion within the cells and ducts) are shaded obliquely.
  d, After vagus stimulation. Granules stain *red* with eosin, but the contents of most of the vacuoles in the cells and the secretion in the large duct stain *orange*.
  e, Vagus stimulation. Some vacuoles stain red; others orange.
  f, Weak stimulation of vagus.
    1 c.c. of juice in one hour and ten minutes.
    Empty open vacuoles are seen, which communicate with intercellular spaces.
  g, Action of secretin.
    The juice in the duct stains *red* with eosin, like the granules. There are no vacuoles which stain with orange.

(Babkin, Rubaschkin, and Sawitsch, 1909. Figs. 1, 3, 5, 16, 18, 21, and 22 in order.)

with secretin. Some of these figures are reproduced in monochrome in Fig. 92 (see description of figure).

If the juice secreted under natural conditions contained active trypsin, it is difficult to understand the use of the production of enterokinase in the intestine. No doubt, however, the flow of water through the cells might carry away zymogen material before it had been worked up by the cell and this would require activation.

It is not only glands with visible secretion that are under the control of the nervous system, but also those of *internal secretion*. The fact has been shown especially in the case of the adrenals. When the splanchnic nerves are excited in any way, there is an output of adrenaline into the blood, which produces the various phenomena due to stimulation of the sympathetic, such as rise of blood pressure, etc. (see Asher, 1910; Elliott, 1912, etc.).

THE SECRETORY PROCESS

It seems evident that there are two kinds of processes, or rather two factors, at work; one concerned with the transfer of water, together with certain solutes already present in blood, the other concerned with the elaboration of new chemical compounds. Whether either of these can be excited without the other, by means of specific nerve fibres or by chemical means, it is at present impossible to state. It is to be remembered that the passage of water in itself would wash out constituents of gland cells previously stored therein, but the results of vagus stimulation on the pancreas indicate that new chemical changes can also be set in action by nerve influence. In the idea of "trophic" nerves, Heidenhain appears to include the function of exciting the formation or replacement of the substances which had been given off from the cells in the process of secretion previously. The vagus effect, described above, suggests rather the setting into play of a chemical change in the products already stored in the cells. Secretin, on the other hand, apparently sets going a process by which water washes out stored substances without change. The prolongation of the period of increased oxygen consumption considerably beyond the actual period of secretion itself, induced by the stimulation of nerves, suggests that the restitution process, by which the cells are restored to a state ready for renewed activity, is an automatic process and controlled by mass action in a reversible system. In the moderated natural process of secretion, such as that of the pancreas induced by the introduction of acid into the duodenum, the fact that signs of fatigue appear in the cells only after very prolonged activity shows that the natural process of restitution keeps pace with the secretory activity of the cells.

On the whole, it appears that the usual process of secretion is somewhat as follows:—During the period of rest, the cells build up compounds which are preliminary stages of constituents of the secretion, which is afterwards set going by excitation, nervous or chemical. The formation of this material is probably a reversible reaction, so that, after a time, further production ceases, owing to accumulation of products. When the gland is excited to activity, a current of water is set flowing through the cell by some means, probably of an osmotic nature and effected by a combination of increased permeability of the outer end of the cell together with splitting up of some substance into smaller molecules. This current of water washes out into the duct the substances of the secretion already stored in the cell, sometimes after they have been further changed by a process which does not take place until the cells are excited to secretory activity. As the stored substances are lost from the cell, there will be a renewed formation to re-establish equilibrium; so that, if the activity is not too violent, there will be a balance between the amount secreted and its new formation. Continuous secretion will thus be possible without fatigue. It will be seen that, on this view, the increased production in the cell of the substances which give rise afterwards to the actual products contained in the secretion is not to be supposed to be under the control of the nervous system or other excitatory influence, but that it is a spontaneous activity of the cell itself, controlled by chemical equilibrium. Thus

the trophic nerves of Heidenhain are not trophic in the sense of presiding over processes of growth of material, but control the changes in the cell which lead to the transformation of stored substance into the specific organic constituents of the secreted fluid.

ANABOLIC OR INHIBITORY NERVES

Under certain conditions, stimulation of the vagus nerve stops a pancreatic secretion in progress, owing to a previous effective excitation, or from injection of secretin. Anrep (1916) has investigated this effect and finds that the explanation lies in a contraction of the ducts. It is not surprising that this should be the case, since, as we shall see in the next chapter, the vagus nerve causes contraction of the intestinal muscle, and the pancreatic ducts are outgrowths from the intestine in development. Anrep placed a portion of the pancreas in a plethysmograph and found that, during the cessation of the outward flow of secretion, the gland increased in volume. This latter fact shows that the secretion continued to be formed, but was unable to escape. After a time, the pent-up juice forces its way out and, as the first drop appears, there is a diminution in the volume of the gland, which returns to its normal volume after the apparent inhibition has ceased. It is of interest, also, to note that there is no evidence in these experiments of the presence of vaso-dilator fibres in the vagus, nor of more than a minimal vascular dilatation in the gland when secretin was used, provided that the preparation was free from depressor substance (probably β-iminazolylethylamine, see Chapter XXIV.).

Bradford (1888, p. 315) considers that the most satisfactory explanation of the curious phenomenon of the "*paralytic secretion*" of the submaxillary gland is to be found in the hypothesis of a special set of fibres in the chorda tympani nerve. Their function is to check or inhibit the spontaneous activity of the gland cells. After section of this nerve, a secretion of saliva commences in about four hours and lasts for some time, the gland undergoing atrophy at the same time. Further discussion of the action of inhibitory nerves will be found in Chapter XIII.

ARTIFICIAL PERFUSION OF GLANDS

How far the chemical mechanism applies to all glands and whether there are any glands devoid of nervous control, it is not as yet possible to state definitely.

Although the latter mode of excitation appears to be complete and adequate in the case of the salivary glands, some observations by Demoor (1911, 1912, 1913) show that, in the absence of certain chemical substances, stimulation of nerves is without effect. If the submaxillary gland is perfused with Ringer's solution, oxygenated, excitation of the chorda tympani nerve still brings about vaso-dilatation, but no secretion of saliva. Under the same conditions, the pancreas produces no juice when secretin is added to the perfusion fluid. At first sight, it might be thought that it is impossible to supply sufficient oxygen merely by solution in a saline solution, considering the large consumption of oxygen by the gland cells. That this is not the cause of the complete absence of secretion is shown, however, by the fact that if a certain amount of serum of the same animal (100 c.c. to 1,400 c.c. of the saline solution) is added, excitation of the chorda tympani nerve produces a flow of secretion, but only for thirty to sixty seconds. It seems probable that the presence of some constituent of the serum is necessary for the due change in permeability of the cell membrane associated with the process of secretion. The comparatively small amount obtained may arise from the previous store in the cells, and the oxygen supply may be insufficient to afford the energy necessary for the new formation of such substances, or only at a minimal rate. Further observations by Demoor are regarded by him as showing that the way in which a nerve acts in exciting secretion is by causing the production of a chemical substance, which itself acts on the cell processes in a way similar to that in which secretin acts on the pancreas. This exciting substance is perhaps of the nature of a hormone and is carried away in the saliva secreted. The evidence consists

in the fact that addition of saliva to the perfusion fluid causes the gland to secrete. The exciting substance is apparently of a compound nature, since, after heating to 65° C., saliva has lost its power of producing secretory activity from rest although it is still capable of accelerating the rate of flow when this has nearly stopped, subsequent to stimulation of the chorda tympani nerve.

The work of Hustin (1912, 1913) on the pancreas is also of interest in this connection. Perfusion with oxygenated Ringer's solution, to which secretin has been added, does not result in secretion; the addition of the blood or certain liquids derived from it, such as hydrocele fluid or lymph, is also necessary. The author concludes that secretin, oxygen, electrolytes, and some substance contained in blood must be simultaneously present. As far as oxygen is concerned, the experiments are conclusive. A mixture of blood, secretin, and saline solution, effective when oxygenated, becomes ineffective when the gases are pumped off. We can readily understand the necessity of electrolytes for maintaining the normal character of the cell processes, and Hustin's experiments show that blood dialysed against isotonic sodium chloride solution is much less effective than normal blood; even dialysis against Ringer's solution seems to deprive it of some important diffusible constituents, since it is not as effective as non-dialysed blood, although greatly superior to that deprived of all its diffusible constituents except sodium chloride.

For example (1913, p. 89), the amount of juice obtained in sixteen minutes by the use of the latter was 0·05 c.c.; if dialysed against Ringer's solution, 0·33 c.c. in fourteen minutes, rather more than seven times as much; with normal blood, 0·70 c.c. in fourteen minutes, or twice as much as the preceding.

The necessity of the presence of some substance contained in blood, other than hæmoglobin, as carrier of oxygen, is not so satisfactorily shown. Washed red corpuscles were found to answer the purpose of the whole blood; although one experiment was performed with a solution of hæmoglobin, which was found ineffective, it must be noted that the material used was a dried preparation by Merck, which probably consisted of methæmoglobin and could not, if so, act as an oxygen carrier. The evidence that certain tissue extracts and lymphatic fluids do not owe their favouring property to their being better oxygen carriers than the saline solution is not sufficient. Moreover, it was found impossible to separate any constituent from these liquids which was able to take the place of blood. The explanation of the process, on the lines of the Bordet-Ehrlich theory of hæmolysis, does not throw much light on its actual nature.

ELECTRICAL CHANGES

A fairly considerable amount of work has been done in connection with the difference of potential found, on stimulation, to occur between that end of a gland cell which is in relation with the duct, or free surface, and that end in relation to the blood supply. The cause of this phenomenon has not yet been made out, but there are one or two points in the process which have a bearing on the questions before us.

Although it had been known for many years that the various glandular tissues of cold-blooded animals, and also the sweat glands of the mammal, gave rise to electrical changes on excitation, it was not until 1885 that it was possible to investigate the different effects in the salivary glands produced by different nerves from this point of view. In that year, in conjunction with Bradford, I was able to show that the potential difference between the hilus of the gland and the opposite surface, that is, between the duct and the surface of the cells turned towards the blood vessels, is of the opposite sign when the chorda tympani nerve of the dog is excited to that when the sympathetic nerve is excited. If the curves of Fig. 93 are consulted, it will be seen that the former is accompanied by a large secretion of saliva, which follows a course very nearly parallel to the electrical change, whereas the latter, of the opposite sign and much smaller, results only in the formation of one drop of saliva. The support which these two opposite effects give to the hypothesis of two different kinds of nerve fibres

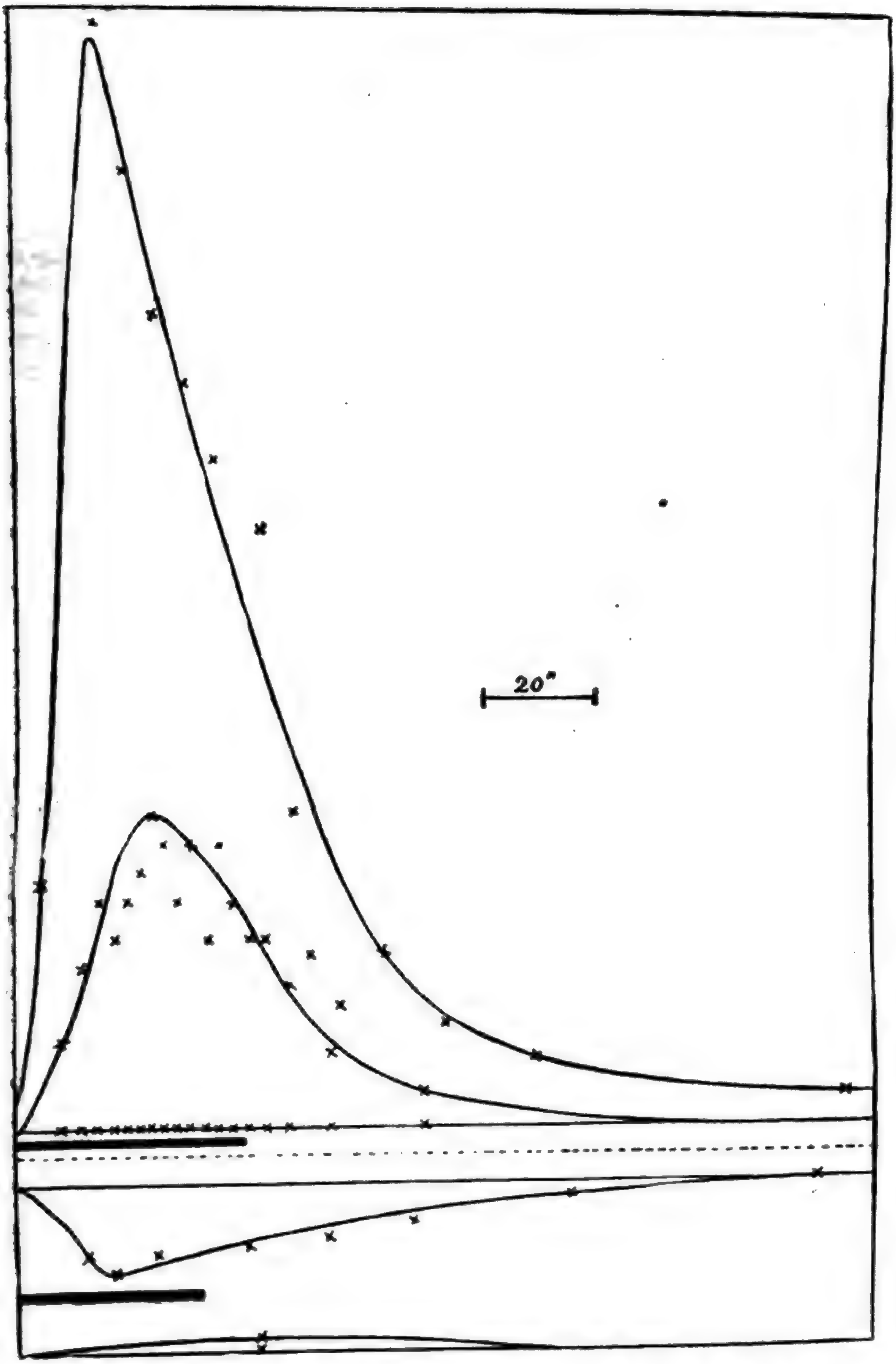

FIG. 93. ELECTRICAL CHANGES IN THE SUBMAXILLARY GLAND OF THE DOG.

Upper set of curves (above dotted line)—chorda stimulation. Period of stimulation marked by thick black line.

Top curve—galvanometer deflection.

Bottom curve—rate of secretion, deduced from the intervals between the drops of saliva marked on the line above the stimulation signal.

Lower set of curves—sympathetic stimulation.

Top curve—galvanometer deflection. Note that it has an opposite direction to that given on chorda stimulation.

Bottom curve—rate of secretion (approximate). Only one drop obtained.

(From data by Bayliss and Bradford, 1885.)

is obvious. Just as the sympathetic fibres are known to require a larger dose of atropine in order to paralyse them than the chorda fibres do, so the electrical change from the latter nerve was abolished by a much smaller dose than that from the sympathetic. But this refers only to that part of the electrical effect from the chorda which is of opposite sign to that of the sympathetic. After a dose of atropine sufficient to paralyse the "secretory" fibres of the chorda, excitation of this nerve gave a small electrical effect of the *same sign* as that from the sympathetic. This effect, normally, is swamped by the much larger opposite one and is, no doubt, due to fibres of the same kind as those which preponderate in the sympathetic. That vaso-motor effects are not concerned in the phenomena is shown by the fact that the electrical changes are abolished by atropine, which does not affect the vascular ones. In the cat, as was shown by Langley (1878), both nerves produce a watery secretion and, accordingly, we find that the electrical change from both is of the same sign as that of the chorda in the dog, but is usually followed by one of the opposite sign.

We therefore drew the conclusion that the electrical change of the sign of the typical chorda effect in the dog is due to the flow of water (together with salts of the blood) and that the other one is connected with the elaboration of the specific organic constituents of the saliva.

Further evidence of the same kind was given by the later experiments of Bradford (1887). Two experiments are of particular interest (pp. 92, 93). The sympathetic in the dog was being excited, giving the usual scanty viscid secretion, with the usual small electrical change. Suddenly a large electrical change of the opposite sign appeared and, coincidently, a copious secretion of watery saliva. In the second experiment the chorda had been stimulated at intervals for an hour and a half. After such treatment, as Langley showed (1889), and as would not be unexpected, since both nerves act on the same cells, stimulation of the sympathetic is apt to give a watery secretion for a time. This was the case in Bradford's experiment, but the watery secretion appeared only after a long latent period, during which the electrical effect was of the usual "sympathetic" sign. As soon as the watery secretion appeared, there was a change in the sign of the electrical effect. After a period of rest, the sympathetic failed to give the watery secretion and the usual "sympathetic" electrical effect reappeared.

The possible causes of these changes will be best appreciated after Chapter XXII. has been read. That the chorda effect is not due to mere flow of liquid along the ducts is shown by another experiment of Bradford's (p. 98) in which clamping of the duct had no effect on the electrical change. Removal of the clamp, after stimulation had been stopped, produced no electrical effect, although a free flow of saliva took place along the ducts. The electrical change is therefore due to phenomena in the cells themselves.

The corresponding changes in the sweat glands (Hermann and Luchsinger, 1878, 1), in the frog's skin (Hermann, 1878) and tongue (Hermann and Luchsinger, 1878, 2) may be mentioned, since they are easily observed.

PRODUCTION OF LYMPH

This phenomenon, as due to increased osmotic pressure in the fluid of the lymph spaces, on account of the diffusion into them of the small molecules of the products of metabolism of the active organ, has been described above (page 165).

The detailed observations of Bainbridge (1900) on the submaxillary gland should be consulted.

ADAPTATION

The possibility of increased production of an appropriate enzyme, in response to the stimulus of a particular article of food, has occurred to several investigators and positive results are said to have been obtained.

Careful testing by subsequent observers, however, showed the presence of unsuspected sources of error. The only case in which any satisfactory evidence exists is that of the increased amyloclastic action of the saliva, as described by Lovatt Evans (1913, 1). Carbohydrate food only had this effect and mere chewing, without swallowing, is ineffective. The simplest explanation is that a

chemical substance of the nature of a hormone is produced by the action of the carbohydrate on the mucous membrane of the stomach, similar to the secretin of the pancreatic mechanism.

THE KIDNEY

Owing to the peculiar arrangements present, special description of the mechanism of this organ is necessary.

We have seen that its activity is confined to the separation of substances which already exist in the blood, with the exception of hippuric acid, and even in this case the chemical change merely consists in the combination of glycine with a benzoyl group, both supplied by the blood.

We have also discussed the function of the glomeruli and come to the conclusion that the liquid leaving their capsules is a filtrate from the blood, having the same composition minus the colloids. The urine as it leaves the kidney, however, is much more concentrated and, as we have also seen, the concentration does not affect all the constituents equally. The problem now before us is the way in which this change is effected as the glomerular filtrate passes along the tubules, which consist of a series of tubes lined with cells of various structure.

To understand the evidence on the question, a knowledge of the structure of the kidney is necessary. This can be obtained from Cushny's book (1917, pp. 1-14) or from the article by Metzner in Nagel's Handbuch (1907, 2) and it must be assumed in what follows here. Fig. 94 will serve to give a general idea of the arrangement of the tubules.

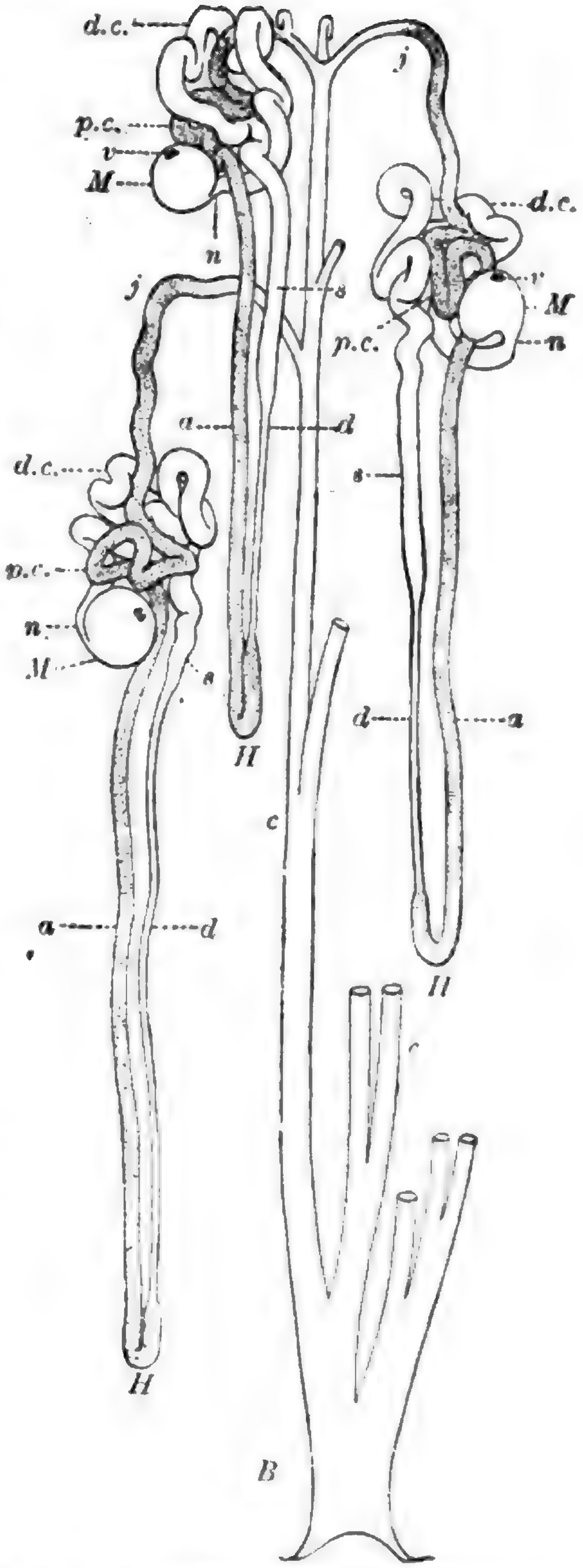

FIG. 94. THE STRUCTURE OF THE KIDNEY. (Huber.)

M, Malpighian capsules containing glomeruli.
V, Entrance of blood vessels into glomerulus.
n, Neck.
d.c, Distal convoluted tubules, arising from malpighian corpuscles.
s, Spiral tubule.
d, Descending limb of Henle's loop.
All the above are left white.
H, Bend of the loop.
a, Ascending limb of Henle's loop.
p.c, Proximal convoluted tubule.
j, Junctional tubule.
These last four parts are grey.
c, Collecting tubule.
B, Duct of Bellini, receiving a number of collecting tubules and opening into the cavity of the pelvis of the kidney.

In the higher animals the function of the kidneys may be said to be of two kinds. In the first place, non-volatile products of metabolism, which are useless or injurious, have to be removed. In the second place, the osmotic pressure of the blood has to be kept constant. This osmotic pressure is due chiefly to the salts, so that the excretion of salts must be increased or diminished, according to the amount taken in with the food, and that of water adjusted in accordance with that taken or lost in ways other than by the kidney. In the lower animals, where the osmotic pressure of the body fluids is that of the solution in which they live, the first function is the chief or only one, so that we will take this first.

We have found sufficient evidence to show that the first stage is a mere filtration from the blood. It is clear that this, if copious enough, would be able to get rid as far as necessary, of all the non-colloidal metabolic products. But, since these are present in very small concentration in the blood, a large amount of water must be filtered with them.

In the case of water animals, this would not be a serious matter. In the fish, *Lophius piscatorius*, Denis (1913) found that the urine in the bladder had a specific gravity of 1·016 and contained only 0·083 per cent. of total nitrogen, but 1·08 per cent. of chlorine. Contrast these figures with those of one of the higher land vertebrates. In man, the percentage of nitrogen is about 1, and that of chlorine about 0·6 per cent. It is obvious that the sea fish has no need to be careful as regards water and chlorides.

In land animals, where water is often difficult to obtain, its loss would be serious to the organism. Salts are also of importance, so that we find that arrangements have been evolved to diminish losses of both kinds. According to the theory put forward by Ludwig (1844), water is absorbed by the cells lining the tubules, as the dilute glomerular filtrate passes over them. The evidence for this must be examined. Further, we have to remember that mere concentration by removal of water will not account for the fact that the concentration of urea goes up very much more than that of sodium chloride, to take the two chief constituents. To do this, either sodium chloride must be absorbed, or urea must be excreted in the tubules. It will be noticed that these two compounds represent two distinct classes of substances which are present in the glomerular filtrate, which is an indiscriminate mixture of all the diffusible substances in the blood. Urea represents the various metabolic products which require removal as far as possible; sodium chloride represents valuable food-stuffs, inclusive of glucose, which should not be lost more than is avoidable and are present in the glomerular filtrate because they cannot help being there, if one may use the phrase. Sodium chloride itself is probably chiefly of importance for the maintenance of the correct osmotic pressure of the blood. This could be done by other salts, but, as we have seen above, those of sodium are the least toxic. Where they are not to be obtained from earth or sea, there is great desire for them, especially in animals taking a diet in which vegetable matter preponderates, since the food does not contain sufficient. The tubules of an ideal kidney, therefore, would absorb, together with water, the useful substances of the filtrate, leaving the metabolites untouched, or even adding to them by active secretion.

In contradistinction to the theory of Ludwig, Bowman (1842), while also regarding the glomerular function as that of filtration, believed that the cells of the tubules secreted the specific contents, such as urea, uric acid, etc., while the filtrate itself, which would be much less copious than that required by Ludwig's theory, contained only water and salts. On p. 75, Bowman speaks of the "escape of water" from the blood, and, from the description given, it seems evident that he regarded the process as a filtration effected by the blood pressure. In any case, the total process must involve work on the part of the cells, since the osmotic pressure of the urine is higher than that of the blood.

Absorption of Water.—If we calculate the amount of glomerular filtrate which must be concentrated in order to give the daily output of urea, as is done by Starling, we find that 28 litres of water must be reabsorbed by the tubules from 30 litres of filtrate. While it is not impossible for so large a quantity of fluid to be filtered by the glomeruli, it seems a wasteful process. On the other hand, if we confine our attention to the sodium chloride, and assume that the excess of urea is secreted by the tubules, only 6 litres of filtrate would be necessary, since 1 litre of blood contains about 2·7 g. of sodium chloride and 15 g. are excreted daily. These 6 litres would only need concentrating down to 1·5 litres. It seems to be forgotten by some opponents of the reabsorption of water, as is pointed out by Cushny, that the cells of the tubules are not comparable to those of a secreting gland which elaborate new substances, and that their function consists in the separation of urea, etc., from the blood. Since, therefore, the urea is only present in a very small definite amount in

the blood, the quantity of liquid to be dealt with by the cells of the kidney is the same, whether it comes to the cells from the blood or from the lumen of the tubules.

Perhaps the following *à priori* considerations may assist the argument. If there is no absorption of water by the tubules, it is necessary to assume that not only are urea and similar metabolites secreted by the tubule cells, but also useful substances like glucose and sodium chloride. Now it is difficult to understand why a wasteful, or at any rate useless, process should have been produced in the course of evolution. If we confine our attention to the higher land animals, it might seem an inefficient process to filter off water and solutes from the blood, only to be in great part reabsorbed. The ancestral excretory organs, however, were probably merely filters, like the glomerulus, and the process was a satisfactory one, since there was no need to preserve either water or salts on account of their abundance in the ocean. As regards organic, diffusible food materials which would escape with the filtrate, they might be kept back in great part by adsorption on colloidal surfaces in the cells of the organism. In the course of evolution on land, the saving of water and salts became more and more advantageous, so that the power of reabsorption began to show itself.

We must not forget, moreover, that the filtration process involves no expenditure of energy on the part of the kidney itself, however large the amount filtered. The energy comes from the heart and is but a small fraction of that used in other ways.

Ribbert (1883) believes that he has positive evidence of the absorption of water by the tubules in the results of removing the medulla of the rabbit's kidney, which takes away the greater part of the tubules. It was found that a much more dilute urine was excreted. But the kidney is a very sensitive organ, and the procedure a somewhat violent one, so that too much stress must not be laid on these experiments.

Absorption of water, in the mammal entirely performed in the renal tubules themselves, appears to take place also in the cloaca, or posterior part of the alimentary canal, in the bird. Sharpe (1912) finds that the urine is a clear liquid in the ureter and only attains its well-known semi-solid nature after leaving the ureter.

If we grant the process of glomerular filtration, and the evidence for this is overwhelming, the fact that the urine contains a larger percentage of sodium chloride than the blood does, is an indirect proof of absorption of water. For, as we shall see later, there is every reason to believe that no secretion of sodium chloride occurs in the tubules.

So far, then, we may state what appears to be the most probable view thus: The glomeruli filter from the blood sufficient fluid to contain the whole of the sodium chloride excreted and probably more; part of the water together with a part of the valuable solutes, such as sodium chloride, glucose and amino-acids, is reabsorbed in the tubules.

Absorption of Solutes by Tubules.—In the frog, owing to the fact that the glomeruli are supplied by blood directly from the aorta, while the tubules are supplied from a separate renal portal vein, it is possible to investigate the two systems separately.

I do not propose to describe the earlier experiments of Nussbaum and others, since they were to a certain extent inconclusive, on account of neglect of the fact that, while the renal portal blood supplies the tubules alone, the arterial blood from the aorta, after passing through the glomeruli, supplies the tubules with oxygenated blood, so that cutting off the glomerular circulation at the same time caused death of the tubules from asphyxia. Those interested will find a description of the experiments in the work of Starling (1920), or of Metzner (1907, 2).

The later experiments of Bainbridge, Collins, and Menzies (1913) have brought out some points of interest, to which brief reference may be made. The urine of the frog is normally of a lower osmotic pressure than the blood, or, if the kidney is perfused with Ringer's solution, the salt concentration of the urine is *lower* than that of the Ringer's solution. This state of affairs is brought about by the tubules, since, when they are poisoned, the urine is always isotonic with the solution perfused, that is, it is a pure glomerular filtrate. Now this activity of the tubules may be due either to secretion of water or to absorption of salts. No evidence could be obtained of the former except, perhaps, under the influence of some diuretic agent such as urea. Sodium chloride must therefore be absorbed. The frog, being essentially a water animal, is under no necessity of hoarding water and, in fact, it has been stated that the urine secreted in twenty-four hours may exceed the total

weight of the body. It would seem possible, then, that the whole of its excretory products could be got rid of by mere filtration; but it is important that the valuable substances, like sodium chloride and glucose, also in the filtrate, should be retained.

Experiments made by Cushny (1901) point in the same direction. In the later stages of the diuresis brought about by injection of a mixture of sodium chloride and sulphate, the proportion of chloride to sulphate in the blood was 0·493 to 0·191, whereas in the urine it was 0·094 to 2·0. The sulphate is much less readily absorbed by the tubule cells than the chloride is, as by cells in general, and it is evident that the fact favours the reabsorption of the valuable chloride. It is possible that the foreign sulphate may actually be excreted by the tubules, but there is no direct evidence of the fact. During the maximum of diuresis, the concentrations of the two salts in the urine approach much more closely to those in the blood, although that of the sulphate is higher than in the blood, while that of the chloride is lower. It is clear that the faster the liquid passes along the tubules, the less opportunity is there for the activity of the cells of the tubules to effect changes in its composition, so that the more rapidly the urine is produced, the more nearly is it isotonic with the blood. It is important to notice that, in Cushny's experiments, the percentage of chloride in the urine was never higher than in the blood. It would appear from some experiments by Loewi (1902) that mere diffusibility is not the only controlling factor when poisonous salts are concerned, since sodium iodide is excreted as effectively as sodium sulphate.

Cushny also performed some experiments in which the kidney was caused to secrete under an increased pressure in the ureter, so that the glomerular filtrate remained longer in contact with the tubules. The results showed a greater absorption of sodium chloride than of sulphate and urea. Of course, the total amount of filtrate is less under the increased ureter pressure, so that one can only compare the proportions of the different constituents and the experiments do not show that there was in fact *any* absorption of sulphate or urea.

If an animal receives no sodium chloride in the food for several days, the serum still contains nearly the whole of its normal amount, but the urine practically none. Very nearly the whole of that filtered through in the glomeruli must be reabsorbed in the tubules.

As already pointed out, the filtration process tends to cause a loss of food materials, so far as these are non-colloidal, as indeed those of the blood are. Although a part of these may be held in adsorption equilibrium, even glucose itself, as pointed out above (page 57), a certain quantity must escape in proportion to the amount of the filtrate. In fact, small amounts of glucose and amino-acids are normally present in the urine. Nishi (1910), however, brings evidence that there is absorption of sugar in the tubules of the cortex. Even when excess of glucose is present in the blood, it is found that the medulla of the kidney contains none, although it is present in the cortex. If diuresis is produced, glucose is present in both parts. The obvious explanation of the results is that most of that present in the glomerular filtrate is reabsorbed in the tubules, except when the current is too rapid to allow sufficient time. Some experiments by Basler (1906) support this view. Sugar solution was run into the ureter of one side under a pressure of 26 mm. of water and was found to be present in the urine of the opposite side.

In experiments of this kind, however, it must be remembered that unless we assume complete impermeability of the tubule cells to the particular substance in question, diffusion must take place to some extent, if the concentration is greater in the lumen of the tubules than in the blood vessels.

For the reason last mentioned, most of the earlier experiments with dyes are capable of interpretation either on the hypothesis of absorption or of secretion. This objection does not seem to hold for those of Ghiron (1913), who injected a small amount of aniline-blue or Congo-red into a vein, while observing with the microscope the surface of the living kidney of the mouse (for the method, see Ghiron's paper of 1912). It was seen that a pale blue or red glomerular filtrate first appeared in the convoluted tubules. This would have the same concentration in dye as that of the blood, so that no dye would pass through the cells of the tubules by mere diffusion, since the concentration would be the same on both sides. But it was seen that the border of the cells next the lumen was the first to become filled with particles of dye, which gradually passed towards the side of

the capillaries. So that the cells evidently absorbed material from the lumen and passed it back to the blood.

The Normal Process.—We arrive then at the following conception of the normal process of renal activity in the higher land animals, as was sketched in outline above. By a retention of the pure filtration process of the lower animals, a filtrate is first made, which contains all the non-colloidal constituents of the blood in the same concentration as therein. But, if this were to be sufficient to carry away the whole of the waste products, which are present in very low concentration in the blood, an enormous loss, both of water and of valuable constituents, would be entailed. To meet this, a mechanism has been developed, by which not only a great part of the water is reabsorbed, but also a large proportion of the valuable salts, such as sodium chloride, and also organic food-stuffs, such as glucose and amino-acids.

It is to be noted that the increase in osmotic concentration, hereby produced, requires the expenditure of energy. This must be afforded by oxidation processes in the living cells of the tubules, the mechanism of which is still unknown.

We have hitherto referred to the absorption of water and of solutes as if they were carried out separately, but Cushny (1917) has shown how much more satisfactorily the whole of the phenomena connected with renal activity can be explained, if we suppose that the fluid actually absorbed has the composition of Ringer-Locke's solution, that is, a fluid containing the normal salts of the plasma in the same proportion as therein, together with the glucose, amino-acids and other valuable diffusible constituents. Excretory products, such as urea, together with foreign salts and other foreign crystalloids, are refused absorption by the cells of the tubules. In other words, these cells have developed the capacity of taking up a particular fluid which corresponds to the useful part of the blood, minus its colloids. The constituents of this fluid are present in the same concentration as in normal blood. This is the distinction made by Cushny between the "Threshold and No-threshold" substances. The former are returned to the blood, the latter escape by the ureter. If the plasma contains too much glucose or chloride, the tubules only return the optimal or threshold concentration, and the rest is excreted. If the glomerular filtrate is too dilute owing to the taking up of water by the blood, "the subtraction of the optimal solution leaves the excess water in the urine."

It is important to note, as Cushny remarks (1917, p. 48), that "the absorption in the tubules is independent of any discrimination, for the fluid absorbed is always the same, whatever the needs of the organism at the moment." But we see, at the same time, how the constant composition of the blood is ensured.

Another point to be remembered is that "the presence of any unabsorbable substance in the fluid passing along the tubules limits the absorption of fluid, for it offers osmotic resistance," which increases until the cell activity is unable to overcome it. The urine can never, therefore, exceed a certain concentration, which differs in different animals.

The reader may have noticed that no further mention has been made of the possibility referred to on page 354, namely, that urea might be excreted into the glomerular filtrate as it passes along the tubules. Although this view is widely held, the evidence for it has never been great, and Cushny (1917, pp. 58-74), after subjection of this evidence to careful criticism, has come to the conclusion that the results can be better explained in other ways than by secretory activity. He brings further direct evidence, showing that "the excretion of urea ceases at the same time as that of water, and that the cells of the tubules are unable to accumulate it either in their interior or in the lumen in the absence of a flow of urine."

The question of the reaction of the urine is discussed in Cushny's monograph (pp. 165-173). It is shown how it can be explained by the presence of hydrolyzed salts, such as phosphates, in the glomerular filtrate. The free base is absorbed by the tubule cells, while the undesirable acid is rejected.

Further details of the way in which other phenomena connected with renal activity can be explained on the basis of Cushny's "modern view" must be obtained from the book itself. The special case of hypotonic urine is treated on pp. 143-145. This view, while being considerably less complex than others, is nevertheless able to explain all the facts.

THE NERVOUS MECHANISM OF THE KIDNEY

Since the rate of filtration from the glomerulus depends on the difference between the pressure of the blood in it and the pressure outside, it is clear that any process increasing the difference will increase the rate of flow. Rise of general blood pressure, produced by means to be described in Chapter XXIII., is one of these. Dilatation of the arterioles of the kidney on the heart side of the glomeruli themselves is another means, and, clearly, a combination of the two would be most effective. Conversely, a diminution of general blood pressure, or a constriction of renal arterioles, decreases the rate of flow. The kidney is, in fact, copiously supplied with vaso-constrictor nerves, and to some extent with vaso-dilator nerves, so that the requisite mechanism is not wanting.

We have seen further that the cells of the tubules intervene by active processes requiring the consumption of energy, so that it does not seem improbable that excitatory nerves may exist. Histologists have described nerve fibres ending in the cells of the tubules (see especially the work of Smirnov, 1901, one of whose figures is reproduced in Fig. 474 on p. 374 of Schäfer's "Essentials of Histology").

Certain experimental evidence has been brought forward by Rohde and Ellinger (1913) that the splanchnic nerve contains fibres which inhibit the activity of the tubule cells. The chief fact in support of this view seems to be that the diuretic effects of section of the renal nerves, due in the first place to removal of tonic vaso-constrictor impulses, lasts for several months, by which time it is supposed that the renal arterioles have recovered from the immediate effect of the section. It is to be remembered that vaso-constrictor reflexes are probably being sent to the intact gland during the time of observation, which are the cause of a diminished secretion on this side; on the side of the section, of course, they would be absent. Some other evidence, with regard to the solid constituents of the urine, seems to me to be explicable by the vaso-motor change, without the necessity of assuming secretory nerves. Asher and Pearce (1913) believe that they have evidence that there are secretory nerves to the kidney contained in the vagus nerve, but the evidence that all vaso-motor action was excluded is not altogether satisfactory. Some observers had previously stated that this nerve contains inhibitory fibres for the secretion of urine (see Bradford, 1889, p. 395). Bradford himself was unable to find any vaso-motor fibres in the nerve (see Cushny, 1917, pp. 10 and 11). Pearce (1915) finds that the vagus does not affect the gaseous metabolism of the kidney.

An interesting morphological point was made out by Bradford (1889) in his investigation of the nerve roots by which the renal nerves leave the spinal cord. The area is a very extensive one, from the 4th thoracic to the 4th lumbar, although the largest number are contained in the 11th, 12th, and 13th thoracic. This long area is of interest in connection with the ancestral origin of the kidney from a series of segmental organs extending over a considerable number of segments.

Diuretics.—All substances, such as salts, sugar, etc., which raise the osmotic pressure of the blood, bring about the passage of water from the tissues into the blood and thus decrease the osmotic pressure of the colloids of the blood. The pressure necessary to separate the glomerular filtrate is thus reduced, or, if it remains constant, the rate of filtration is increased. The presence of a foreign salt, such as sulphate, which is not absorbed, holds back water. In the experiments of Barcroft and Straub (1910), it appears at first sight as if sulphate excretion required more oxygen consumption, but, if the figures are examined (see Cushny, 1917, p. 37), it turns out that more sulphate was actually eliminated in the period with Ringer solution and no increase of oxygen consumption, though the percentage was less. The increase in consumption of oxygen seems to be due to the extra work of concentration.

Urea causes diuresis by dilatation of the renal arterioles, without any considerable effect on the general blood pressure. The diuretic effect of *glucose* lasts longer than its effect on the concentration of the blood plasma ("hydræmic plethora"), so that it seems to bring about a local dilatation of the kidney arterioles, in addition to its dilution effect.

It has been shown by Cushny (1902) that if the increased blood flow through the kidney, produced by injection of 3 per cent. sodium chloride, be brought back to its initial rate by an adjustable clamp on the renal artery, the diuresis ceases; so that the vascular change is the responsible factor and no specific action on the cells is present.

Certain evidence indicates that such specific diuretics as the purine derivatives,

caffeine, etc., may have a paralytic effect on the absorption by the tubules. We have seen above that, although an animal may be deprived of chlorides in the food, the blood continues to preserve nearly its normal concentration (0·7 per cent.) in sodium chloride, while the urine may contain as little as 0·08 per cent., owing to the almost complete reabsorption of this important salt by the tubules. Under these conditions, if one of the diuretic drugs referred to be administered, the amount of the urine is increased and the sodium chloride goes up to 0·64 per cent., as shown by Pototsky (1902). Such an increase is considerably greater than would be accounted for by the lessened absorption of sodium chloride on account of the more rapid passage along the tubules.

An interesting specific diuretic action is exerted by a hormone formed by the *pituitary gland*, as described by Magnus and Schäfer (1901) and by Schäfer and Herring (1906). Extracts of this organ cause a rise of blood pressure together with vaso-dilatation of the kidney and increased flow of urine. The diuresis and kidney dilatation last longer than the rise of general blood pressure, so that there must be a specific effect on the kidney itself. The fact is suggestive in connection with the view taken by Gaskell (1908, pp. 215 and 321) of the origin of the pituitary body from the coxal glands of the invertebrate ancestor, which were excretory in function and remain the chief excretory organ in Limulus. One is reminded also of the effect of saliva in producing activity of the submaxillary gland, as described by Demoor (1913).

CERTAIN PECULIAR FORMS OF SECRETION

Some special products of secretory activity may be referred to briefly in order to show the great variety of products which different organisms are able to manufacture.

Acid and Alkali.—In the large mollusc, *Dolium galea*, a kind of salivary gland exists, which produces sulphuric acid of the strength of 4 to 5 per cent. (Preyer, 1866), apparently used for attacking the calcareous shells and spines of starfish, and other echinoderms used as food. The same purpose is probably served by the large percentage of aspartic acid produced by some related molluscs. It seems desirable that the fact of secretion of 5 per cent. sulphuric acid should be re-investigated.

The production of hydrochloric acid in the stomach, of decimolar or even higher concentration, has not yet received a satisfactory explanation. It is clear that a large amount of osmotic work must be done in the process, and it is difficult to suggest a possible chemical reaction by which it might be obtained under the conditions compatible with cell life. Miss Fitzgerald (1910) gives some hypotheses on the question. In a mixture of chlorides and acid phosphates, there will be present both $H^{\cdot}$ and Cl' ions, so that if the cell membrane is permeable to these and not to other ions of the cell contents, it seems possible that the secretion may be explained, although the hypothesis is doubtful.

Koeppe (1900) shows, that if the cell membrane is permeable to cations ($H^{\cdot}$ and $Na^{\cdot}$), impermeable to anions (Cl'), acid might appear outside the cell (Findlay, 1905, p. 50). Although this appears to be the usual state of the cell membrane as regards its permeability (Bayliss, 1919, 3, p. 83), the experiments of Benrath and Sachs (1905) do not confirm the hypothesis as regards gastric juice.

The fact of the production of an acid reaction when an electro-negative colloid, such as arsenious sulphide, is thrown down by neutral salts of barium, etc., as mentioned above (page 94), may have some connection with the phenomenon. It is, perhaps, most likely that the surface action of colloids may ultimately afford a satisfactory explanation, when taken in connection with special arrangements of the cell membrane as regards permeability.

Similar remarks apply to the production of a secretion of alkaline reaction, such as the pancreatic juice.

The cuttle-fish, *Sepia*, as is well known, produces an inky fluid to cover its retreat from enemies. The pigment contained in this secretion is used by artists as a pleasant warm black or brown paint. It is one of those black or brown compounds known as melanins, and, according to von Fürth (1903, p. 372), is formed in the cuttle-fish by the action of an oxidising enzyme, tyrosinase, on tyrosine.

Poisons.—Many interesting substances of this class are to be met with, sometimes produced for the capture of food, sometimes for defensive or offensive purposes against enemies. The mason wasp injects, with its sting, a toxic substance into the nerve ganglia of its prey, spiders. This produces paralysis, while the spiders still remain living and ready for food when wanted. The wasp, in fact, deposits an egg in proximity to the paralysed spider, so that, when the grub hatches, it finds fresh food, living but powerless, ready for it to consume (see Warburton's "Manual," 1912, p. 124).

Henze (1913) shows that the poison used by cephalopods to paralyse their prey, especially crabs, can be extracted by alcohol from the posterior "salivary" glands. The active substance is found to be para-hydroxy-phenylethylamine, which was shown by Barger and Walpole (Barger and Dale, 1910, p. 31) to be produced from tyrosine, by removal of CO_2, in the putrefaction of meat. The chemical relationship of this substance to adrenaline is of interest in view of the production of this latter in the organisms. Thus:—

Para-hydroxy-phenylethylamine. Adrenaline.

It is also interesting to note that Sepia, one of the cephalopods, makes use of tyrosine in another way, to form the black pigment of its inky secretion, as mentioned above.

Salivary glands in the snakes have been differentiated into organs for the formation of extremely powerful poisons. These are of two main classes: some, such as that of the Australian black snake (*Pseudechis porphyriaca*), investigated by C. J. Martin (1894), act on the blood, causing intravascular clotting; some of this class also contain hæmolytic substances. The other class, typified by the Cobra, causes paralysis of respiration (Lamb, 1903), but Cushny and Yagi (1916) show that the only action of cobra venom is like that of curare to paralyse motor nerve endings, and that other effects are indirect. For other snake poisons, see Frazer and Gunn (1909 and 1912).

The meaning of the enormous variety of toxic and other *alkaloids* produced by *plants* is very difficult of explanation. It would seem that, if their presence were merely to avoid being eaten by animals, one or two distasteful substances would have sufficed. It may be that they are in many cases, as it were, accidental by-products of metabolism, although the possibility of some hitherto unknown action on nutritive processes must not be forgotten.

Hirudin.—It has long been known that the blood sucked by the leech into its alimentary canal remains liquid, and it was found by Haycraft (1884) that there are certain unicellular glands, close to the mouth of these animals, which secrete a substance which has the power of depriving blood of its coagulating property. This it does both when injected into the blood vessels of a living animal, or when added to the blood *in vitro*. The leech appears to benefit from the arrangement in two ways: there is no risk of blocking of the fine incision made by its teeth in the skin of the animal attacked and from which it is sucking blood, and the blood in its alimentary canal is naturally more accessible to the action of enzymes than if solidified.

The substance, which can be obtained in solution by extracting the heads of the leeches with water, either directly, as in Abel's method (1914), or after drying with alcohol, as in that used by Haycraft, has been of great service in experiments where it is necessary to collect blood from the veins of organs or to measure its rate of flow. This applies both to organs *in situ* and to artificial perfusions. The blood rendered non-coagulable by this means appears to be more normal than if defibrinated by whipping; rabbits are killed by injection of

their own defibrinated blood, but are unaffected by extract of leeches. A dry commercial preparation of great activity, known as "hirudin," which is made by the method described by Franz (1903), is much used. Franz regards the active constituent as being a kind of albumose. Abel finds that it is in colloidal solution in water.

Silk and the similar substance of spiders' webs are very interesting products of secretion. They are of protein nature and are formed in the liquid state by special glands. The liquid is forced through fine apertures and rapidly sets in contact with air. By opening up the silkworms, a considerable quantity of the liquid secretion can be obtained, which can then be used for making fibres of greater thickness than those made by the insects themselves. These threads are of great strength, and are valuable for fish lines, ligatures, etc.

The Gas Bladder of Fishes.—Since the substance of which the body of fishes is composed is of a higher specific gravity than that of sea water, it is obviously of advantage to them to possess a float, containing gas which, present in the appropriate amount, will reduce their weight to that of an equal bulk of water, thus removing the necessity of muscular movement in order to keep themselves from sinking. An organ of this kind actually exists in the teleosts. But it is clear that the gas will be compressed as the fish sinks, thus becoming of a greater specific gravity, and more must be produced to restore proper compensation. Conversely, when the fish rises again, the gas will expand and displace other tissues, as in fact happens when deep sea fish are brought to the surface rapidly. In some fish, there is a duct to the exterior, via the œsophagus, which can be opened to allow of escape of gas; and in others, where the duct has become solid, in a special region, the "oval," the wall of the gas bladder has the power of absorbing the gas. This oval can be shut off from communication with the gas bladder when not required. At first sight, it might seem strange that the gas is found to consist almost entirely of oxygen, but if it has to be secreted and absorbed, the advantages are obvious. Oxygen can easily be obtained from oxyhæmoglobin and can be used up either by combination with reduced hæmoglobin or by oxidation of some reducing substance. Woodland (1911, 1 and 2) has made an interesting investigation of the structure and physiology of the gas bladder, and the reader is referred to his papers, which contain also a list of other papers. A remarkable vascular organ is found in the course of the blood vessels supplying the gland in the gas bladder which secretes oxygen. This organ is what is called a *rete mirabile*, in which the artery divides into a number of fine arterioles, which lie closely, side by side, with the corresponding finely divided veins carrying blood from the gas gland. These vessels do not join each other, but allow of free interchange of diffusible constituents of the blood, and have, obviously, an important function in relation to the secretion of gas. It would seem that some chemical substance must be produced in the gland cells, which is not desirable in the general circulation. As this substance returns in the veins, it diffuses out into the blood of the arterioles in the rete and is, for the most part, sent back to the gland. It is probably something which enables oxyhæmoglobin to give up its oxygen readily; that there are substances of this kind will be seen in Chapter XXI. It is not a hæmolysin, since there is no evidence of the presence of such a substance, nor is there hæmoglobin in the cells of the gland itself. The gas must therefore be given off by the corpuscles in much the same way as to other tissue cells. The preparations of Woodland show that the gas gland possesses large cells, similar in appearance to those of a typical secreting gland, and, according to Bohr (1894), the vagus nerve supplies secretory fibres, since, after section of the intestinal branch of this nerve, no further secretion of gas takes place, even when the bladder is emptied of gas, a procedure always resulting normally in renewed formation of oxygen.

The protozoan, Arcella, forms bubbles of gas and raises itself to the surface of water by this means. According to Bles (1910), these bubbles consist of oxygen. The stimulus to secrete the oxygen bubble appears to be, curiously enough, the want of oxygen in the depths of the pond water; the animal thus floats itself to the surface. In order to sink again, the animal must absorb the oxygen, since it cannot escape to the air, owing to the shell on the animal.

Luminous Substances.—There are many organisms known which are capable of secreting substances which give off light. Very little is known of the chemical nature of the reaction concerned, but it is evidently an oxidation process of some kind, since the luminosity disappears in the absence of oxygen. According to the work of Raphael Dubois (1913 and literature cited therein), there are two substances concerned, neither of which is luminous alone. The one is of the nature of an oxidising enzyme, or peroxidase, the nature of which we shall have to discuss in Chapter XX. This can be replaced by solution of a permanganate, or by some other oxidising enzyme. It is called "luciférase." The substance oxidised is called "luciférine"; its chemical nature is unknown, but it appears to have some of the properties of proteins. A remarkable fact about the light produced is that the radiation contains only a very small percentage of the longer wave lengths, known as heat rays, and is almost entirely composed of "light" rays. It has hence been designated "cold light" and indicated as the ideal illuminant. Further details will be found in Chapter XIX.

The mollusc, *Pholas dactylus*, which bores its way into hard mud on the sea coast, is frequently to be found and has a brightly luminous secretion.

The work of Molisch (1904) on luminous bacteria will be found of much interest. The article by Mangold (1910) on the production of light by organisms may also be consulted.

Electrical Organs.—In the electrical fish, Malapterurus, found in the Nile and known to the ancient Egyptians, the electrical organ is evidently developed from skin glands. We have seen that the process of secretion is accompanied by electrical changes and it is curious to note how this has been made use of for the purpose of defence and perhaps of benumbing prey. In other electrical fish, the organ seems to have been formed from muscular tissue and will be referred to in Chapter XXII.

SUMMARY

In a general way, all living cells may be said to give off to the surrounding medium products of the chemical reactions taking place within them. But the name of secretion is especially given to those cases in which the products are made use of for purposes of importance to the organism as a whole.

Under the name are also included processes in which the function of the cells is to separate from the blood products of the metabolism of the organism as a whole. These waste products would be deleterious if allowed to accumulate, and there are arrangements produced in order to reject them to the exterior of the organism. This process is sometimes called "excretion," and is the particular function of the kidney, although the epithelium of the alimentary canal takes part in the excretion of foreign substances under certain conditions.

Secreting organs, or glands, may either discharge their products by means of a special channel, the duct, into a cavity such as the alimentary canal, which cavity is, in a sense, outside the organism itself; or their products may diffuse into the blood vessels and in this way affect distant organs. Glands of this latter kind are known as those with internal secretion.

The products of glands with external secretion, such as the pancreas, are given out, for the most part, dissolved in water, so that the first problem is the way in which the cells produce a current of water through their substance in order to wash out, as it were, the chemical compounds which they have formed.

The filtration of pure water from a solution of the molar concentration of blood is impossible by pressures directly available in living organisms. If, however, the liquid to be filtered off consists of blood minus its colloids only, the arterial pressure is higher than the osmotic pressure of these colloids and can filter off a solution of this kind. The process actually occurs in the glomerulus of the kidney.

Since it is found that the pressure under which secretion is possible is higher, in some cases, than that of the arterial blood, osmotic forces are indicated as the

source of the energy required. Certain possibilities are indicated in the text as to the way in which these osmotic forces are available. But, in the end, the production of osmotically active material must be ascribed to what, in our present ignorance, we call "protoplasmic" activity, by means of which the chemical energy derived from oxidation of food is converted into the various other forms of energy required.

In most cases, the process of secretion is found to be accompanied by the disappearance of certain granules, "zymogen," from the cells of the gland. These granules appear to be a stage in the formation of the constituents of the secreted fluids.

The production of osmotically active substances, together with changes in the permeability of the cell membrane, appear to be the chief factors in the actual process of secretory activity.

The first stage in the formation of urine is the filtration in the glomerulus of a liquid which is identical with blood-plasma minus its colloids; so that the rate of secretion under a given pressure is inversely proportional to the osmotic pressure of these colloids in the blood, and if the blood pressure is lower than this osmotic pressure, no filtration takes place.

The work done in secretion is, in the main, of two kinds, although the ultimate source of both lies in chemical energy utilised in cell processes. The work done in producing a secretion of higher osmotic pressure than the blood can be calculated by the method given in the text. That done in the various chemical reactions can only be estimated approximately by the amount of oxygen consumed. The work in glomerular filtration is not derived from the kidney itself, but from the contractions of the heart muscle.

The measurements of oxygen consumption give some indications as to the nature of the cell process. The increase is found to take place, not only during the secretory process, but for some time afterwards. This obviously means that energy from some reaction is being stored up during rest and in a form available for the next period of activity. There does not appear to be any storage of "intramolecular" oxygen, since the secretory activity is greatly dependent on the supply of oxygen in the blood at the time of secretion itself.

Glands are set into activity either by means of chemical substances circulating in the blood, such as drugs or the natural "hormones" such as secretin, or by the agency of nerves supplying the gland cells. This statement does not exclude the possibility that the final link in the chain of excitation processes may be the same chemical substance in all cases.

Evidence, taken as a whole, indicates that there are two kinds of nerve fibres to glands; one kind, the "secretory" of Heidenhain, presides over the secretion of water, together with diffusible substances present in blood, and must, therefore, affect both permeability of cell membrane and the osmotic pressure of cell contents. The other set, "trophic" of Heidenhain, are concerned with the production of the specific solid constituents and have little or nothing to do with the phenomena connected with the production of a flow of water.

The combination of various facts indicates that during rest gland cells form, by means of reactions which are reversible, certain substances which are preliminary stages of the constituents of the actual secretion formed on stimulation. When the gland is excited to activity, a current of water is set flowing through the cell by a combination of increased permeability of the outer end of the cell with the splitting up of some cell constituent into smaller molecules and thus raising the osmotic pressure. This current of water washes into the duct various substances stored in the cell, sometimes after these have been changed by the excitation process, before being given off. As these substances are removed, further formation takes place by the cell reactions in order to re-establish equilibrium.

The results of experiments on artificial perfusion of salivary glands show that

some constituent of blood is necessary for the production of secretory activity by stimulation of nerves. In the case of the pancreas, the combination of at least three agents is necessary for secretion: oxygen (in greater amount than can be dissolved in saline solutions), electrolytes, and secretin. Whether any other constituent of blood, such as protein, is necessary is uncertain. It is obvious that prolonged activity is only possible when materials for the formation of the constituents are supplied.

The electrical changes of gland cells when excited are of two kinds with opposite sign. That associated with the secretion of water has an opposite direction to that associated with the formation of the organic solids which are characteristic of the secretion.

The kidney requires special consideration. The glomerular filtrate, as it passes along the tubules, suffers concentration owing to the cells of the tubules absorbing from it a fluid having the composition and concentration of the normal optimal Ringer-Locke's solution, containing, in addition to glucose, any other food material, such as amino-acids, present in the glomerular filtrate. In other words, they have developed the capacity of refusing admission to waste products, together with toxic substances foreign to the organism, and taking up, in their optimal concentration, those constituents which are of value, although unavoidably present owing to their non-colloidal nature. This is done by the tubule cells under all circumstances, without discrimination, but it is clear that the result must be to maintain a constant composition of the blood.

Most diuretic substances act by diminishing the osmotic concentration of the blood colloids; some, by a specific action of some kind, perhaps by decreasing absorption of water, etc., by the tubules.

The formation of certain special secretory products is briefly discussed in the text. These are acid and alkali, sepia, poisons, hirudin, silk, oxygen, luminous substances, and electrical charges.

LITERATURE

General.
Heidenhain (1880). Babkin (1914).

Structure of Gland Cells.
Metzner (1907, 1).

Osmotic Work Done.
Von Rhorer (1905).

Oxygen Consumption.
Barcroft and Piper (1912).

Salivary Glands.
Langley (1898).

Secretion of Urine.
Metzner (1907, 2). Cushny (1917).

CHAPTER XII

DIGESTION

The great majority of the materials taken in as food by animals require treatment of some kind in order to enable them to be carried by the blood or other fluids to the various organs requiring them.

In the green plant, the food-stuffs do not require treatment of this kind, but, even here, stored products such as starch and protein require the action of certain enzymes before they are again available for the use of the cells, or to be conveyed to distant growing parts.

In the animal, the conversion of food-stuffs into diffusible or assimilable substances is known as *digestion*, and is carried on in the alimentary canal, chiefly by means of enzymes secreted into the cavity by the various glands opening into it or situated in its walls. In the digestion of certain food-stuffs, especially that of cellulose in the herbivora, bacteria play an important part.

To describe the great variety of digestive mechanisms met with in the animal kingdom would take far more space than is permissible here. It may be said in general that the object of these mechanisms is to ensure the effective action of the digestive enzymes, and the due absorption by the blood or lymph of the products of their activity. Details of these mechanisms may be found in the article by Biedermann (1911).

INTRACELLULAR DIGESTION

In the unicellular organisms the whole process takes place within the one cell. The food-stuffs are taken in, a vacuole containing liquid is formed around them, and the necessary enzymes secreted into this vacuole. Material undigested is extruded through any part of the cell. Although raw starch appears difficult of attack by protozoa, the fact that boiled starch is hydrolysed shows that they possess an amylase. They also store glycogen, a fact indicating the reversible action of an amylase. But, in any case, protein appears to be their chief food, obtained in the main from bacteria and algæ. According to the work of Nierenstein (1905), the reaction of the contents of the food vacuole is at first acid to neutral red, and subsequently becomes alkaline. During the acid period, no digestion takes place, but the reaction seems to be connected with the killing of the bacteria taken as food. The actual digestion occurs during the alkaline stage. Mouton (1902) prepared an enzyme from a large number of amœbæ, and found that it would not attack living bacteria, but dead ones rapidly, so that the preliminary killing in the acid stage is of importance. The enzyme acted best in a medium alkaline to litmus, but acid to phenolphthalein, that is, faintly alkaline; it produced tyrosine and is, accordingly, to be considered as a trypsin.

Phagocytosis.—We find the process of intracellular digestion, similar to that of the amœba described above, still present in the amœboid cells of the higher multicellular organisms. The leucocytes of vertebrates require special attention. In these, Metschnikoff has shown that the taking up of living bacteria plays an important part in the defence against infection by micro-organisms. He called the process "phagocytosis." It is not confined to certain leucocytes of the blood, but is manifested by large cells found in the peritoneal cavity and elsewhere. The reader may consult the book by Metschnikoff (1901) for further details. The process of phagocytosis has been referred to previously (page 3) and Ledingham's work mentioned. According to this observer, the bacteria in the blood are not

taken up by pseudopodial processes of the phagocytes, since in the circulating blood current they are spherical cells; when a bacterium comes into contact with a phagocyte, it is engulfed, killed, and digested. It will be clear that any influence which makes adhesion more certain on chance contact will increase the number of bacteria taken up, as will also, as Ledingham points out, any agglutination of the bacteria into clumps, since a large number will be taken up at a chance encounter instead of a single one. There does not seem to be any necessity for the assumption of such ill-defined chemical substances as "opsonins," which have been supposed to make the bacteria attractive or "tasty" to the phagocytes.

The process of phagocytosis is also met with in the absorption of larval organs, such as the tail of the tadpole. It plays a part in the formation of bone; and certain cells in the liver, Kupffer's "star-cells," are phagocytes. In the extraordinary "disruption" of internal organs that takes place in the metamorphosis of the fly, wherein nearly the whole of these organs become broken up preparatory to the formation of the new organs of the adult, phagocytes play a considerable part.

Digestion in the Sea Anemone.—The process here deserves a little consideration, since it forms a kind of transition to that of the higher multicellular animals. Although there is what seems like a gastric cavity, no one has yet succeeded in showing the presence therein of any secretion with digestive properties. Nevertheless, animals of considerable size are actually digested by the anemone and in fact by a somewhat remarkable process. There are long filaments in great number attached to the septa in this gastric cavity and these wind themselves all over the body of the animal taken in and into all depressions and cavities in it. This they do by a kind of pseudopodial movement, probably conditioned by surface tension, like that of the Amœba. Where the cells covering the filaments are in contact with food material, they secrete digestive enzymes limited to the area of contact. These enzymes cause the tissues attacked to break up and the fragments are then taken in by the cells and dealt with further by the ordinary process of intracellular digestion. It appears that the enzymes can diffuse for a short distance, since food wrapped up in filter paper is digested, provided that the paper is moistened with meat extract. The presence of some chemical stimulant in the meat causes the secretion of enzymes in the cells of the mesenterial filaments at the places where they come into contact with it.

GENERAL PLAN IN THE HIGHER ANIMALS

The alimentary canal may be said to consist of a long tube, with dilatations in places. In this tube the food is subjected to the action of a series of enzymes. The dilated sections are capable of being shut off from the neighbouring sections by means of rings of muscle, the sphincters, so that the food shall not be passed on to the next section prematurely. As a rule, arrangements exist by which the secretion of the digestive juice in a particular section is brought about by the presence of food in the preceding section, so that no delay in the process occurs. The food is kept in movement by muscular contractions of the walls of the canal and passed on by similar movements. These movements are partly provided for by nerve centres in the wall of the canal itself, but are under the control of the central nervous system. As regards the sphincters, it is usual to find that they close by a nervous reflex when a certain amount of material has been passed through to the next section.

Each of these factors requires a little more detail. In the following description the state of affairs as fully developed in the case of one of the higher vertebrates, man or the dog, is taken as typical; in lower animals, vertebrate and invertebrate, various simplifications are to be found, as well as special additions for particular purposes.

MOVEMENTS

If the small intestine is cut out of an animal such as the rabbit, cat, or dog, and immersed in warm, oxygenated Ringer's solution, or better, in Ringer-Tyrode's solution, it is seen to exhibit a series of rhythmic contractions, which travel as

waves along the muscular coats. But another kind of movement can be produced by mechanical stimulation, such as that which the presence of food would afford. The nature of this movement was demonstrated by Bayliss and Starling (1899) in the intestine *in situ*, but separated from any connection with the central nervous system. It can also be obtained in the excised gut, under favourable conditions, and most readily in the colon of the rabbit. A gentle pinch at any spot causes a cessation of the rhythmic contractions in the region just *below* the place excited, together with a diminution of the state of moderate tonic contraction in which the intestinal muscle normally exists, upon which the rhythmic contractions are superposed. The word, "below," of course, refers to the normal direction of the movement of the contents of the intestine, and would perhaps better be called, "in front of." Coincidently with this relaxation in front of the point excited, there is an increased tonic contraction, with greater vigour of rhythmic movements, *above*, or behind the place excited; these facts are illustrated by Fig. 95. To this phenomenon, which is common to the alimentary canal from the lower part of the œsophagus to the rectum, in a more or less well-marked manner, we gave the name of "The Law of the Intestine." It is clear that it is what would happen if the intestine were distended at any spot by the presence of food, and that it would result in the moving on of the contents. In fact, a lump of cotton wool smeared with soft soap and inserted into the front end of a loop of rabbit's intestine (the colon works best), immersed in warm saline, is gradually passed on and escapes at the lower or posterior end.

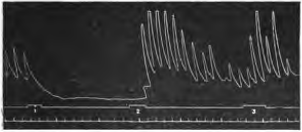

FIG. 95. THE LAW OF THE INTESTINE.—Contractions of the jejunum traced by a balloon in the lumen.

At 1, pinched gently about an inch *above* the balloon. Immediate inhibition of rhythmic contractions below, with relaxation of tone.
At 2, while still quiescent, a gentle pinch about half an inch *below* the balloon. After some six seconds sudden recovery of tone, with series of strong rhythmic contractions.
At 3, similar pinch during normal contractions. Greatly increased strength of contractions.

(Bayliss and Starling, 1899, Fig. 10.)

Cannon (1912) suggests that a better name for the reaction in question would be the "myenteric reflex," and the name has the advantage of being applicable to the whole alimentary canal. On the other hand, Gaskell (1916, pp. 78 and 121) has suggested how it may be brought about without the assumption of reflex capabilities of a peripheral nervous system, which are unknown elsewhere in the vertebrate. Auerbach's plexus has been shown to be necessary for the manifestation of the phenomenon. But this plexus consists of the cell stations of the vagus nerves only. In certain invertebrates, there are neurones of which the axons branch into two fibres, one, owing to the manner of its termination, causing *contraction* of a muscle fibre, the other causing *inhibition* of another muscle. If then the vagus neurones of Auerbach's plexus send an excitatory fibre backwards and an inhibitory one forwards, the stimulus to the cells by the distension of food would produce contraction behind and inhibition in front of the mass. Dogiel (1889), in fact, has shown that each neurone of Auerbach's plexus has two axons

which have different destinations (see Garrey and Moore, 1915). The mechanism will be better understood after Chapter XIII. has been read. The part played by Meissner's plexus is unknown.

It seems clear, however, that some further regulation of the passage of food is required to prevent its being hurried along too rapidly; even a means of sending it backwards and forwards. The muscular contractions causing movement onwards are generally known as "peristalsis" and those in the opposite direction, "antiperistalsis," but Cannon (1912) suggests, as a better terminology, the name "diastalsis" for the forward moving wave, controlled by the myenteric reflex and preceded by inhibition; and "ana- and cata-stalsis" for the rhythmic waves of upward or downward movement, which are present independently of the myenteric reflex and not preceded by a wave of inhibition. These movements take place when the myenteric reflex is put out of action, as it can be by the influence of the central nervous system, as we shall now see.

There are two great nerves which control nearly the whole of the alimentary canal; the vagus, which is the ancestral motor nerve for the whole of the gut, except the two extreme ends; even in the higher mammals it continues to act as motor nerve as far as the large intestine, remarkable as it may seem that a cranial nerve should have so posterior a distribution. Gaskell, in fact (1908, pp. 446-454), draws interesting conclusions as to the origin of vertebrates from the innervation of the alimentary canal.

Eduard Weber (1846) in his classical article in which he describes the discovery of the inhibitory action of the vagus nerve on the heart, also (p. 49) describes a remarkable effect of the same nerve in the Tench, where it produces a quick contraction of the intestinal muscle, "like skeletal muscle."

The second great nerve supply is from the sympathetic system and contained in the splanchnic nerves. This is inhibitory. In Fig. 96 a tracing is given which shows how the rhythmic contractions are stopped and the tonus abolished when this nerve is excited. It is, as a rule, impossible to obtain the myenteric reflex unless the splanchnic nerves are cut, owing to the inhibitory control they have over its manifestation.

A curious fact in connection with the vagus is that its motor effect on the small intestine is preceded by an inhibitory one, and that the motor effect is not shown until after the nerve has been subjected to a series of periods of stimulation (see Fig. 97). Gaskell's view of the nature of the "law of the intestine" makes both the inhibitory and excitatory components functions of the vagus.

The more posterior part of the large intestine has its motor nerve supply from the pelvic visceral, or autonomic, nerves.

The best means of investigating the normal movements of the alimentary canal in digestion is that introduced by Cannon (1897 and 1902). The animal, or man, is given bismuth subnitrate, an insoluble, inert powder, mixed with the food; by this means the contents of the alimentary canal are made opaque to the Röntgen rays and their shadows can be watched on the fluorescent screen. It has been made out that the food, having arrived into a particular section, say the stomach, is imprisoned therein for a time by closure of the sphincters. During this imprisonment it is thoroughly churned, backwards and forwards, until the enzymes have had time for their work, and the products of their activity, if absorbed in this part, have been taken into the blood and lymph. The process of churning, whose mechanism can be observed best in the small intestine, does not consist in a true antiperistalsis, preceded by a wave of inhibition, but by a series of local contractions dividing the contents into separate masses at different places in turn and thus pushing them upwards and downwards.

It has been shown by Serdjukov (see Pavlov, 1901, p. 187) that the pyloric sphincter of the stomach opens at intervals to allow a portion of the contents to escape into the duodenum; as soon as acid is present therein, the pylorus closes by a nervous reflex, so that only a small amount of food is allowed to enter the duodenum at one time.

The churning movements are not observed in excised intestine, and they may possibly be in some way controlled by the central nervous system (see the author's "Introduction to General Physiology," p. 80, and Cannon's monograph, 1911).

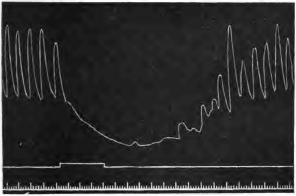

FIG. 96. EFFECT ON THE INTESTINE OF STIMULATION OF THE SPLANCHNIC NERVES.—Cessation of contractions and relaxation of tone.

(Bayliss and Starling, 1899, Fig. 16.)

An interesting point with reference to the *ileo-colic sphincter* is described by Elliott (1904). The splanchnic nerve, on stimulation, causes its muscular cells to contract instead of inhibiting their tone, as it does in the neighbouring parts of the small and large intestines. Adrenaline has the same effect. The significance of this fact in regard to the origin of the alimentary canal will be found in Gaskell's book (1908, p. 449).

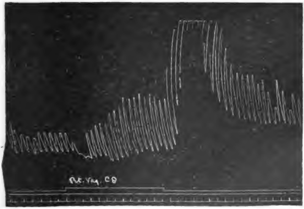

FIG. 97. EFFECT ON THE INTESTINE OF STIMULATION OF THE VAGUS NERVE.—Ninth period of stimulation. Slight preliminary inhibition, followed by powerful contractions, beyond the limit of the recording instrument.

(Bayliss and Starling, 1899, Fig. 25.)

A fact which indicates the dependence on the central nervous system of those co-ordinated movements of the intestines which are not included in the myenteric reflex is described by Gottfried Boehm (1912). Cannon had shown that the food, after passing into the colon, is kept there for a time and churned backwards and forwards in order to ensure absorption of water and any products of digestion still left. Boehm found that excitation of the vagus nerve increased the vigour of the backward movements.

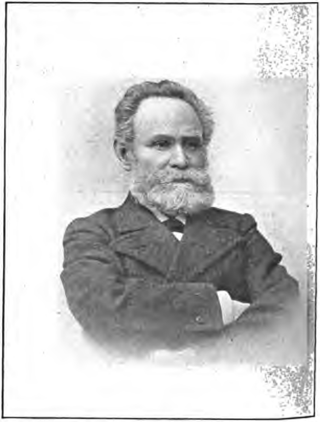

FIG. 98. PORTRAIT AND SIGNATURE OF IVAN PETROVICH PAVLOV.

THE SECRETION OF THE DIGESTIVE JUICES

The food, as it passes along the alimentary canal, is subjected to the action of a series of fluids containing enzymes, whose action will be described in the following section of this chapter.

The modes in which the secretion of these juices is set going have been worked out almost entirely by Pavlov and his co-workers and will be found described in his book (1901). When references are given to this in the following pages, it is to be understood that they apply to the English edition. A portrait of Pavlov will be found in Fig. 98.

Fig. 99. Portrait of Regnier de Graaf.—From his book (1677).

Fig. 100. Title page from the book by Regnier de Graaf (1677).

On the desk are displayed the syringes which he invented and used for anatomical injection of arteries. There is also a uterus with ovaries and Fallopian tubes. It is difficult to say whether the human body is supposed to be in process of dissection, or whether the female figure is intended to be understood to call attention to the value of the author's discoveries for the purpose of curing disease. In the foreground are the bodies of various animals used, together with a living dog provided with a pancreatic and a salivary fistula, each with a receptacle for collecting the secretion.

It will be noticed that that method of investigation which consists in the establishment of openings, or fistulæ, in various parts of the alimentary canal plays a large part in work on the digestive processes. It is interesting to note that, so far as we know, Réné de Graaf (1677) was the first to make use of this method. In Fig. 99 his portrait is reproduced. There are several points of interest in the

title page to his book (Fig. 100). These will be found in the description; what concerns us here is the dog in the foreground with salivary and pancreatic fistulæ. The work of Spallanzani on digestion should also be mentioned (see Foster, 1901, pp. 213-216).

For the various operative procedures required, the articles by Pavlov (1902) and by London (1910) may be consulted.

Saliva.—This is the first fluid met with and it is produced even before the food enters the mouth. This "psychical secretion" is caused by reflexes through sight, smell, and so on; the mouth "waters." The taste of the food in the mouth causes renewed secretion.

The Gastric Juice.—When the food enters the stomach, it finds that gastric juice has already been secreted. This first secretion is psychical and depends greatly on the appetite with which the eating of food is approached. It is produced before food actually enters the mouth and, in fact, the mere presence of food in the mouth, without appetite, does not excite secretion. When, therefore, Macbeth wishes for his guests that "good digestion" may "wait on appetite," he is merely expressing a physiological fact.

If solid food is introduced directly into the stomach through an opening, a gastric fistula, unknown to the dog under experiment, no secretion is produced for an hour or more. Mechanical stimulation of the mucous membrane of the stomach is also ineffective.

The efferent nerve through which the glands of the stomach are excited is the vagus.

Certain chemical substances introduced into the stomach produce a secretion. According to Edkins, as mentioned already, a hormone, analogous to the pancreatic secretin, is produced from the mucous membrane of the pyloric portion and carried in the blood to excite the glands of the fundus. The experiments of Pavlov in connection with the chemical mechanism were performed mainly on dogs provided with a miniature stomach, separated from the main one by an ingenious operation, which is a greatly improved form of a similar one done by Heidenhain (see p. 13 of Pavlov's book). This miniature stomach was found to serve as a sample or indicator of all that proceeded in the main stomach.

It was found that meat juice or Liebig's extract caused secretion; but no result was obtained from raw egg-white, nor from starch nor fat. That the mechanism is a chemical one and not nervous is shown by the fact that Liebig's extract and similar substances are effective after the vagus nerves have been divided.

The Pancreas.—The mode of excitation of the pancreas was described in the previous chapter. We see that the acid gastric contents, when they arrive in the duodenum, give rise to the production of secretin, which excites the pancreas. Whether the vagus takes any part in the normal process we have seen to be doubtful. If it does so, there is a possibility of "psychical" secretion from appetite, in addition to the effect of escape of the acid "psychical" gastric juice passing into the duodenum.

The Bile is another important secretion poured into the intestine. Its function will be discussed presently. We have seen that the same acid extract of duodenum which excites the pancreas also causes the secretion of bile, so that the acid contents of the stomach when they arrive in the duodenum cause also a secretion of bile. We have no evidence of a nervous control over the liver, with the exception of vaso-constrictor nerves to the branches of the portal vein. For the general conditions of the secretory mechanism, see Okada (1915).

Succus Entericus appears to be excited, in any particular part of the intestine, by the presence of pancreatic juice in the parts preceding this one.

THE CHANGES IN THE FOOD

We may now proceed to describe the changes which the food undergoes in the several parts of the alimentary canal.

Since most food is taken in the form of more or less solid masses, a means of *disintegrating* it is clearly of advantage for the ready access of enzymes. Most animals possess some means of doing this. Masticating apparatus, such as teeth

and strong jaws, are common. In those birds which eat hard grains there is a powerful muscular organ, the gizzard, whose cavity contains small stones swallowed by the bird and acting as mill-stones grinding the food before it is passed on to the stomach for digestion.

In animals which chew the cud, *Ruminants*, the grass, etc., after it has been roughly chewed in its first gathering, passes into a large receptacle, where it undergoes a softening process by the action of bacteria. It is then brought back into the mouth again, bit by bit, and thoroughly masticated before being again swallowed and passed into the digestive stomach.

In some animals, as the crayfish, there is a masticating apparatus in the stomach, which is followed by a kind of filter, so that food is not allowed to pass into the intestine, into which the chief digestive gland opens, until it has been adequately subdivided.

The main purpose served by the *saliva* appears also to be a mechanical one, since in many animals it contains no enzymes. It enables dry food to be readily masticated and swallowed. In animals taking food containing starch, we find that the saliva contains an amylase, sometimes called "ptyalin," which converts starch into maltose and, under favourable conditions, by further hydrolysis to glucose. We have already seen how carbohydrate food, by some chemical mechanism, causes an increase in the amylase content of the saliva in man (Lovatt Evans).

Having commenced with *carbohydrate*, we will follow its progress further, and afterwards that of proteins and fats.

Although ptyalin is a powerful enzyme, it has too little time to do much work while the food remains in the mouth. It is inactive in so strongly acid a solution as the gastric juice, but it has been shown by Grützner (1905) that the food in the stomach does not at once come into contact with the acid secretion, especially that food which is latest swallowed, which lies for some time in the middle and is protected by that first swallowed. So that, if the food be mixed with blue litmus, the centre of the mass in the stomach remains blue for some time and amyloclastic action can proceed. In any case, starch that escapes the action of ptyalin meets with the pancreatic juice in the intestine, and the amylase contained therein effects complete hydrolysis.

As regards other carbohydrates, cane-sugar, maltose, and lactose are hydrolysed by appropriate enzymes formed by the glandular epithelium of the small intestine.

The manner of dealing with *cellulose* is of some interest. An enzyme which acts on cellulose is found in seeds—barley, for example—and has also been observed in the alimentary canal of the mealworm and in the secretion of the "liver" of the snail (Biedermann, 1911, p. 980), which forms hexoses and pentoses from various celluloses. It attacks also mannanes, galactanes, etc., the so-called "reserve" or storage celluloses. But in the higher animals the assistance of bacteria, chiefly in the large intestine, is required. In the ruminants, bacterial action also takes place in the paunch, before the second chewing process. The large cæcum present in those animals which take cellulose in quantities, such as the rabbit, horse, or sheep, will be remembered and the mechanism of the digestion of cellulose must be effective, since it is said that sheep will get fat on blotting paper. Now the difficulty is that bacteria carry the process of destruction too far, producing hydrogen, methane, carbon dioxide, and lower fatty acids. Pringsheim (1912), however, has succeeded in showing that a bi-hexose, together with the product of its further hydrolysis, glucose, is formed as an intermediate product. These sugars were obtained by stopping the fermentation at its height by the addition of toluene. It is uncertain whether the antiseptic acts by destroying the particular organisms responsible for the production of hydrogen, methane, etc., or whether it sets free the cellulose-hydrolysing enzyme from an intracellular condition. It may possibly merely put a sudden stop to all further change, so that a certain amount of intermediate products are, as it were, caught on the way. In any case, the "cello-biose" and glucose were isolated from cultures with filter paper of de-nitrifying, methane-producing, or better, thermophile bacteria. The sugars had been produced from the paper. It is interesting that cello-biose is also hydrolysed by emulsin. There seems to be

no doubt that the glucose is, in great part, absorbed from the alimentary canal before the bacteria are able to complete its destruction.

Absorption of Sugars.—It is a remarkable fact that no absorption of digestive products takes place in the stomach. The chief absorption is done in the small intestine.

Digestion and Absorption of Proteins.—The saliva contains no enzyme which acts on proteins. But in the stomach they are acted upon by a powerful hydrolysing enzyme, pepsin, which acts only in acid solution. Hydrochloric acid is secreted by glands in the wall of the stomach, and is of very wide distribution, being found even in the selachian fishes. It appears that, under usual conditions, pepsin does not carry the hydrolysis beyond the stage of the higher polypeptides, known as peptones. These are not absorbed, but passed on to the duodenum to be acted on further. The acid of the stomach has also a function as an antiseptic; not a very powerful one, however, since we know that certain bacteria, for example the Bulgarian lactic acid bacillus, can be introduced into the intestine by way of the mouth. Acid, in any case, is a very unfavourable medium for the growth of bacteria, and hydrochloric acid in the concentration of that in the stomach kills a large number. We saw that the first stage in the fate of bacteria in the food vacuoles of amœba, or phagocytes, takes place in an acid reaction; but no digestive process commences until the reaction changes to an alkaline one. The acid reaction is associated with the killing of the organisms taken as food.

As the acid contents of the stomach are allowed to pass in small portions at a time into the duodenum, pancreatic juice is poured out by the mechanism already described (page 344), and further hydrolysis of the peptones formed in the stomach, together with that of any unattacked protein, is brought about by trypsin, in an alkaline solution. This alkalinity is not so great as was thought at one time, and as that of the pancreatic juice as it leaves the duct might lead one to suppose. There is considerable neutralisation by the acid of the stomach contents when they mix with the pancreatic juice. In fact, according to Michaelis and Davidsohn (1911), the optimal hydrogen ion concentration for trypsin is 10^{-8} normal, which is only just faintly alkaline to phenolphthalein.

We have already seen that the pancreatic juice does not contain active trypsin, but only its zymogen, and that it is necessary for it to be acted upon by another enzyme, enterokinase, produced by the cells of the small intestine, for conversion into active enzyme. As shown by Mellanby and Woolley (1912), this process of activation has a remarkable time course. It starts slowly and becomes more and more rapid as it proceeds. Whether it has the typical S-shape of the curve of autocatalysis is difficult to make out, but it continues to accelerate in rate until the reaction is practically complete. No satisfactory explanation has yet been given of this phenomenon. According to Vernon (1913), it is due to the production of an unstable substance, which itself acts as an activator, but is rapidly destroyed. Ordinary trypsin, in fact, does not activate trypsinogen.

It is evident that some kind of autocatalysis takes place in the activation of trypsin. It may be that some product is formed by the action of enterokinase, which product has the property of increasing the activity of the enterokinase. Vernon (*Biochemical Journal*, **8**, p. 528) holds that the trypsin, as it is set free, acts upon some precursor with the formation of another enzyme, "deuterase," which itself activates trypsinogen, independently of enterokinase.

Bernard (1856, p. 513) refers to the fact that pancreatic juice is much more active when mixed with the contents of the duodenum. In fact, it does not appear that he had found it, as secreted, to act on proteins to any perceptible extent. His attention was thus directed chiefly to its action on fats and on starch. The facts serve to show Bernard's experimental skill, since it is evident that he had obtained pure juice without contamination.

Trypsin hydrolyses proteins to amino-acids. There are, however, as seems probable, some of the simpler di- or tri-peptides which are not attacked very rapidly by it. These are hydrolysed by the erepsin of the succus entericus, an enzyme which does not act on proteins themselves, or rather only on caseinogen and fibrin, and on these only slowly, but converts peptones and other polypeptides into their component amino-acids. It was discovered by Cohnheim (1906).

London (1906, etc.), using the Pavlov method of fistulæ in various parts of the alimentary canal, has found that proteins are practically entirely converted into amino-acids and absorbed as such in the small intestine. One of these, arginine, a conjugated di-amino-acid, as we have seen, is further hydrolysed by an enzyme, arginase, into urea and di-amino-valerianic acid. This enzyme was discovered by Kossel and Dakin (1904).

Digestion of Fats.—A lipase has been described by some as present in the stomach, but its function is negligible compared with that of the pancreatic juice. The action of this latter is assisted by the bile, which promotes fine emulsification, owing to its power of reducing surface tension, and as we have seen, it also has a direct effect on the activity of the enzyme. The bile also acts as a solvent for the free fatty acids, especially important in the case of the higher ones, such as stearic, etc. By this means, fats are hydrolysed into their constituent fatty acids and glycerol.

Absorption of Fats.—These fatty acids and glycerol are taken up by the cells covering the villi, and, in their interior, are synthesised into neutral fats again, probably by the reverse action of lipase (Hamsik, 1914). In the form of fine droplets, the neutral fats are passed into the central lymphatic space of the villus, and thence in the stream of lymph into the lacteals and thoracic duct and so into the blood stream. In the blood they can be observed by ultra-microscopic methods of illumination as the "blood dust," with its vigorous Brownian movement. It is difficult to see precisely why fats should be hydrolysed only to be resynthesised in the villi, but it must clearly be for the purpose of facility of absorption.

Absorption of Water and Salts.—Water can be absorbed by the intestinal mucous membrane to practically any extent. The regulation of the water content of the organism is carried out by the kidneys. No water is absorbed from the stomach, most in the small intestine and a certain amount in the large intestine.

If it were pure water that is to be absorbed, the osmotic pressure of the constituents of the blood-plasma would suffice to explain the fact; but it actually happens that isotonic saline solutions can be absorbed. Even hypertonic solutions are ultimately absorbed after a preliminary dilution by pure osmotic action. There must, therefore, be some active intervention on the part of the absorbing epithelial cells, by which energy is consumed. This process is thus a kind of inverse of that involved in secretion. It is perhaps most strikingly shown by the fact that the animal's own serum can be absorbed. At the same time, physical factors have considerable effect on the rate of absorption and a discussion of the part played by these factors will be found in the article by Starling (1909, 2).

SUMMARY

In animals, food requires treatment, mechanical and chemical, before it can be absorbed. Digestion mechanisms ensure this, and also provide for efficient absorption of the products.

In unicellular organisms, the solid food particles, mostly living algæ or bacteria, are attacked inside the cell. They are first killed in acid medium, then digested by enzymes in alkaline medium.

Similar statements apply to the phagocytes of the higher, multicellular animals. There is no evidence of prehensile, pseudopodial attack; chance contact with bacteria leads to adhesion and engulfing by the action of surface forces. "Opsonins," as specific chemical entities, in all probability have no existence.

The general plan of the digestive system in the higher animals is a long tube with dilatations in places and arrangements for enclosing food, temporarily, in various sections, in order to enable the enzymes, which are secreted into these sections, to act for a sufficiently long time.

Two kinds of movements are required. One to pass the food along the gut, the other to send it backwards and forwards in a particular section. The former is provided for by a nervous mechanism in the wall of the alimentary canal itself.

This is responsible for the myenteric reflex, or "law of the intestine," which consists in the production of a relaxation, with inhibition of movements, below the spot at which a mass of food is found, and an increase of tone, together with more powerful contractions above the spot, thus moving onwards the contents of the intestine at this spot. This effect can be prevented by a set of nerve fibres, in the splanchnic nerves, arising from the central nervous system. Another set of fibres, in the vagus nerve, produces increased movements. In this way, all kinds of movements are provided for.

The normal movements can be best investigated by the aid of the Röntgen rays.

The secretion of the various digestive juices is excited in two ways—chemical, by the presence of a substance in the blood which has been produced by the action of something contained in the food mass, when it arrives in a particular section of the alimentary canal; or nervous, by reflexes excited by the sight, smell, or taste of food. The relative part played by these two mechanisms changes from the mouth to the large intestine in such a way that the glands nearer the head are more under control of nervous reflexes. It is doubtful whether secretory nerves play any important part, in normal conditions, in the cases of the pancreas, liver, and small intestine.

The food is first disintegrated mechanically, sometimes with the aid of bacteria.

Carbohydrates are then converted into their constituent hexoses or pentoses by a series of enzymes, contained in saliva, pancreatic juice, and succus entericus. Cellulose is usually converted into glucose by the action of bacteria in the large intestine and is probably absorbed in this stage before the bacteria have converted much of it into more degraded products, such as hydrogen or marsh gas. In a few animals, an enzyme capable of hydrolysing cellulose has been found in the secretion of digestive glands.

Sugars are chiefly absorbed in the small intestine; when arising from cellulose, in the large intestine.

Proteins are converted first into proteoses and peptones (higher polypeptides) by the pepsin of the gastric juice, and these into amino-acids and some di-peptides by the trypsin of the pancreatic juice, and finally completely into amino-acids by the erepsin of the succus entericus.

Proteins are not absorbed in the stomach, but their absorption is practically complete by the end of the small intestine.

Fats are hydrolysed in the small intestine by the pancreatic juice and absorbed as glycerol and fatty acids, the latter for the most part in solution in bile. In the epithelium of the villi they are resynthesised to neutral fats, which pass into the lymph of the lacteal system and thence to the blood in a finely emulsified state.

Water and salts are absorbed by the mucous membrane of the intestine. Active intervention on the part of cell mechanisms must be postulated to account for the absorption of solutions isotonic with the blood.

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CHAPTER XIII

EXCITATION AND INHIBITION

When a process of any kind takes place continuously of itself without intervention from without, it is clear that, for purposes of due regulation in the living organism, there must be means of modifying it in both directions; there must be some power of either increasing it or decreasing it according to necessity. Cases of physiological processes of this kind are the muscular coat of the small arteries and other places where we find that kind of muscular tissue known as smooth, pale, or involuntary muscle. This, in its usual state, which may be regarded as "resting," since it is the condition taken on when unaffected by nervous impulses, is in a state of partial contraction or "tone." This tone is capable of being increased by certain nerves supplying the tissue and diminished by another set of nerves. In the preceding chapter we saw how the automatic movements of the intestine can be stopped by the splanchnic nerve and increased by the vagus. For our present purpose it is immaterial whether these movements are due to periodic discharges of nerve cells in Auerbach's plexus, or inherent in the muscle cells themselves, although the work of Gunn and Underhill (1914) shows that the latter is the correct statement. In either case, the responsible cells can be either restrained or excited. Such double effects play a fundamental part in the mechanism of nerve centres, as we shall see later. Perhaps the most striking instance that can be given is that of the heart. As is well known, this organ continues its regular series of beats even when cut out of an animal; but, in its natural state, it can be stopped by the vagus nerve or excited to increased rate and force by the "accelerator" nerves from the sympathetic system.

This capacity of being affected in two opposite directions is not confined to living matter. Consider again our old example of an ester system in equilibrium. As we have seen, if we add more water, there is increased hydrolysis; if we remove water, there is increased synthesis, or diminished hydrolysis, of ester. The effect of a catalyst should also be kept in mind, as consisting merely in the acceleration of the attainment of equilibrium, by addition or subtraction of water; that is, it *increases both* hydrolysis and synthesis; which of these effects will be the more obvious one depends on circumstances. But again, the accelerating action of a catalyst can be itself increased or diminished. Pepsin hydrolyses proteins at the greatest rate in the presence of a certain definite concentration of hydrogen ions; suppose that this concentration is somewhat less than the optimal one, it is plain that we can increase the rate of hydrolysis by adding more acid or diminish it by adding alkali.

The duality of phenomena illustrated in the last example reminds us further of the opposition in general chemical properties between hydrogen and hydroxyl ions. The existence of positive and negative electricity may also be mentioned, although perhaps the final word has not been said on this question.

In discussing the phenomena of metabolism, we saw how two processes might be distinguished, the building up of a complex system or substance of high potential energy, "anabolism," and the breaking down of such a system, "catabolism," giving off energy in other forms. Such a case we saw in the secretion of the salivary glands and shall meet with again in muscular contraction. The tendency of much recent work, however, is to throw doubt on the universality of this opposition of anabolism and catabolism as explanatory of physiological activity

in general, since it appears that many protoplasmic processes may be compared rather to the utilisation of fuel in a petrol motor, where the fuel does not become built up into a chemical complex with the mechanism, but gives up its energy by means of the mechanism. The mechanism acts upon it from without, in a certain sense. Further discussion of this question will be necessary later.

The name "excitation" is usually given to the increasing or setting into action of a process, and that of "inhibition" to the opposite phenomenon of stopping a process or decreasing its activity.

In the strict sense, all living protoplasm is "excitable," that is, it is capable of being affected by external forces, as was clearly pointed out by John Brown (1788, p. 3 of 1795 edition) and, in more detail, by Claude Bernard (1879, 1, p. 242), who defines "irritabilité," which is equivalent to the name "excitability" as used above, as "la propriété que possède tout élément anatomique (c'est à dire le protoplasma qui entre dans sa constitution) d'être mis en activité et de réagir d'une certaine manière sous l'influence des excitants extérieurs."

Nevertheless, it is usually the custom to apply the name especially to such tissues as respond to stimuli by a rapid change of some kind and more particularly to nerve and muscle.

THE PROCESS OF EXCITATION IN NERVE

As animals in the course of evolution increased in size and complexity, means of communication between different parts became more and more necessary. To a certain extent, such intercommunication is effected in a chemical way, through the blood, or similar fluid. But this is not sufficiently rapid for many purposes, the fact of contact of a solid object must be conveyed to the muscles of locomotion, so that the organism may react rapidly enough to avoid it. Hence we find the presence of nerves at a very early stage of evolution of multicellular animals. Even in Cœlenterates, the complexity of the nervous channels is considerable. The effect of something happening at one end of such a thread is conveyed with great rapidity to the other end of the nerve, wherever it may be. Our study of the phenomena of excitation will begin with that of the nerve fibre. In some ways, it is the simplest case; in others, more difficult. Nerve fibres have no other function than that of conveying excitations. When left alone, they are, as far as we know, in complete rest, so that their activity does not require inhibitory influences to quell their state of excitation. When set into activity by some influence, called a "stimulus," the disturbance set up disappears spontaneously after a certain very short time, if the stimulus ceases to act.

If we take what is known as a "nerve-muscle preparation," that is, the gastrocnemius muscle of the frog, with the nerve, the sciatic, supplying it, we find that if we lightly pinch the end of the nerve distant from the muscle, the latter enters into contraction, and, as it seems, simultaneously with the stimulus. Nothing to be seen has happened in the nerve, yet something must have passed along it from the point at which it was pinched, otherwise the muscle would have been unaware of anything having taken place at the other end of the nerve. It is usual to speak of a "propagated disturbance" passing along the nerve, or sometimes a "nerve impulse." But how are we to detect it and investigate it in the nerve itself, apart from the indicating muscle?

The most careful investigation with the most sensitive apparatus has only been able to detect with certainty one kind of change accompanying the passage of the "propagated disturbance," namely, an electrical effect.

The production of heat in any quantity that would have any significance at all is definitely excluded by the experiments of A. V. Hill (1912). By the use of a method by which changes in temperature of six-millionths of a degree could be detected, no effect was obtained by twenty-five seconds continuous stimulation. This result means that a single propagated disturbance does not result in the production of more than $1^{\circ} \times 10^{-8}$ C., that is, a hundred-millionth of a degree. Hill calculates that heat of this amount would be afforded by the consumption of 1 molecule of oxygen by a volume of nerve of 3·7 μ cubic measure,

a volume easily visible under the microscope and, if filled with oxygen at atmospheric pressure, would contain 3×10^9 molecules, since 1 c.c. contains $4{\cdot}5 \times 10^{16}$. This result makes it impossible to suppose that any chemical process resulting in an irreversible loss of energy, such as an oxidation, can be involved, and indicates that a reversible physico-chemical one of some kind is to be looked for. Moreover, we must examine with care statements that have been made as to the production of carbon dioxide by nerve in activity.

Waller (1896) noticed that the action of small amounts of carbon dioxide on nerve was to increase the extent of the electrical changes and that tetanisation had the same effect. The natural conclusion was that carbon dioxide was produced by the nerve in its own activity. Tashiro (1913, 1) brings direct evidence. A very delicate method was used for the estimation of carbon dioxide (1913, 2), dependent on the formation of a film of barium carbonate on the surface of a drop of barium hydroxide solution. The quantities found were, of course, very small, and the possibility that they were merely dissolved in the tissue is not satisfactorily excluded. Small amounts were given off very slowly by the resting nerve. The extra production on electrical excitation would easily be accounted for by the heat resulting from the passage of the current. A nerve trunk consists not only of nerve fibres, but of connective tissue in which are living cells, which contain carbon dioxide from their own respiration, and this would be partly given off in consequence of the heat produced by the exciting current itself. There was a regular falling off in the output of carbon dioxide by resting nerve, even during the time in which there is every reason to suppose that the excitability was undiminished. Killing by steam abolished the power of giving off carbon dioxide, both resting and on excitation; but this does not show that it was a vital phenomenon, since carbon dioxide dissolved in the tissues would naturally be driven off before the observation. The numerical data given throw doubt on the origin of carbon dioxide from the metabolism of the nerve itself, since it would be higher than that of an equal weight of muscle. In the monograph by Tashiro (1917), he does not satisfactorily answer the objections. The only additional evidence brought (p. 40) is that the carbon dioxide given off is not proportional to the length of the nerve traversed by the stimulating current but to that through which the nerve impulse passes. Unfortunately, no experimental data are given and no reference to where they have been published. The fact that less carbon dioxide is given off in hydrogen is not convincing without the information as to how long the nerve had previously been exposed to the current of gas. One of the new results, namely, that ganglion cells do not give off any more carbon dioxide than nerve fibres do, tends rather to confirm my doubt. Riggs (1919) supports Tashiro, A. R. Moore (1919) fails to do so.

FIG. 101. DIPHASIC CURRENT OF ACTION IN OLFACTORY NERVE OF PIKE, AS SHOWN BY THE CAPILLARY ELECTROMETER.

Stimulation with single break induction shock at rise of signal.
Time in 0·025 second.

(After Garten.)

We are thus limited to the *electrical change*. Fortunately, this can be measured with accuracy and its time course determined. For the present, we will omit discussion as to its cause, and limit ourselves to the fact that a spot in a state of excitation behaves as if electrically negative to a spot on the nerve at rest; that is, if the two points are connected to a galvanometer, a current flows through the instrument from the resting to the excited spot, as if the former corresponded to the copper of a Daniell battery and the latter to the zinc. We find that the electrical change set up at one point by a momentary stimulus lasts only for a short time at this point and passes along the nerve, making each point in turn electro-negative to the rest. Suppose that two electrodes lie on the nerve at different points and that a stimulus is applied near the one electrode; the electrical response will consist of a current in one direction as this electrode becomes negative to the more distant one, followed by a current in the opposite direction as the wave, having left the first electrode, arrives at the second, making it negative to the other. If we call the electrodes A and B, we have, first, A negative, B positive, then A positive, B negative. This form of electrical response is called "diphasic" (see Fig. 101). If electrode B is on a spot which has been killed, the wave of negativity, as it is commonly called, disappears in the killed area, so that the only electrical effect seen is that due to the becoming negative of spot A and consists, therefore,

of only the first half, as it were, of the diphasic response; it is, in fact, "monophasic."

We know, then, that a propagated disturbance can be set up in a nerve fibre and we have next to inquire how such a "nerve impulse" is excited for experimental purposes. The agents which do this are known as "stimuli" and, for practical purposes, electricity is the most useful, since its strength can be accurately and conveniently graduated and measured. In the application of a single stimulus, consisting of a definite quantity of electrical energy, we must remember that energy is made up of two factors, quantity and intensity, so that we can make up the same amount of electrical energy by varying the two inversely. Now it was found by Waller (1899) that this is not a matter of indifference. There is a certain definite ratio, different for different excitable structures, and different conditions, such as temperature, at which a smaller quantity of energy will excite than at another ratio, in which either the quantity or the potential is higher or lower. This is called by Waller the "characteristic" number. How is it to be explained? Investigations of this kind can be best made by the use of condenser discharges. When two metallic plates, separated by a non-conductor, are charged to a different potential by connection to a source of electricity, the quantity required to produce a given potential difference between them depends on their size, distance apart and the dielectric constant of the medium between them, as we saw on page 180. This is known as the "capacity" of the condenser. By taking condensers of different capacities and charging them to different potentials, we can obtain all the varieties required. The energy in ergs of the discharge of a condenser is given by the formula, $1/2\ U^2\ C$, where U is the potential difference between the plates in volts and C the capacity in microfarads. The expression, it will be noted, is analogous to the ordinary one for kinetic energy.

When a certain quantity of electricity is discharged through a high resistance, such as a nerve, there is a perceptible difference in the time taken for the discharge, according to the potential at which it commences. The formula expressing this fact is:—

$$E_1 = E_0 \times e^{-\frac{t}{RC}}, \quad \text{or} \quad t = RC \log_e\left(\frac{E_0}{E_1}\right),$$

when there is no considerable self-induction in the circuit.

E_0 is the potential difference between the plates before commencement of discharge.

E_1 is that after the lapse of time, t, during which the condenser has discharged through the resistance, R.

C is the capacity,

and e, the base of natural logarithms.

From this formula it will be seen that, other things being constant, the time taken for discharge is proportional to R or to C.

In the paper by Hermann (1906, p. 554), a series of curves will be found, showing the different steepness of the curves of discharge of condensers of different capacity.

The reader may be reminded that the capillary electrometer, used so frequently in the investigation of the electrical changes of tissues, behaves as a condenser in its time curves of charge and discharge. In the determination of the constants in these cases, as in general, where the process starts rapidly and becomes slower and slower as the final state is approached (Newton's "law of cooling," see above, page 157), it is customary to make use of the time taken for half the process to be completed, since the curve is changing its shape most rapidly at this period. Towards the end, measurements are difficult and inaccurate on account of the slow change. In the case of a condenser charged to a potential of 1 volt, the character of the discharge is given by the time taken to fall to half a volt. In the excitation of nerve, in fact, the steep and only active part of the discharge is well over before this time; slowly changing currents have no exciting effect, as will be seen later.

It appears from Waller's experiments, that there is a particular steepness of curve which produces its effect with least expenditure of energy, and it seems

justifiable to connect this fact with the rate of movement of some constituent of the nerve system, somewhat as a push of a given strength, applied to a resting heavy pendulum, will have a greater effect if the rate at which its energy is imparted to the pendulum coincides with the vibration period of the latter.

In the practical use of the condenser, it is important to remember that the insulation is never perfect, so that if any delay occurs between the charge and the subsequent discharge through nerve, the most effective part of the discharge, namely the steepest fall of potential, will have been lost. For this reason, the best arrangement of the circuit is that given in Fig. 102, ascribed by Hermann (1906, p. 540) to Radaković. The condenser C is connected to a source of adjustable potential through the nerve N. When the key K is closed, a chosen fraction of the potential difference of the battery B is sent into the condenser through the nerve. As long as the key remains closed, the full charge of the condenser is kept up to the potential required, but the moment that the key is opened, discharge takes place through the nerve and the part of the slide wire between A and F. If either the charge or the discharge is not intended to pass through the nerve, a short circuit is made for the time being between D and E.

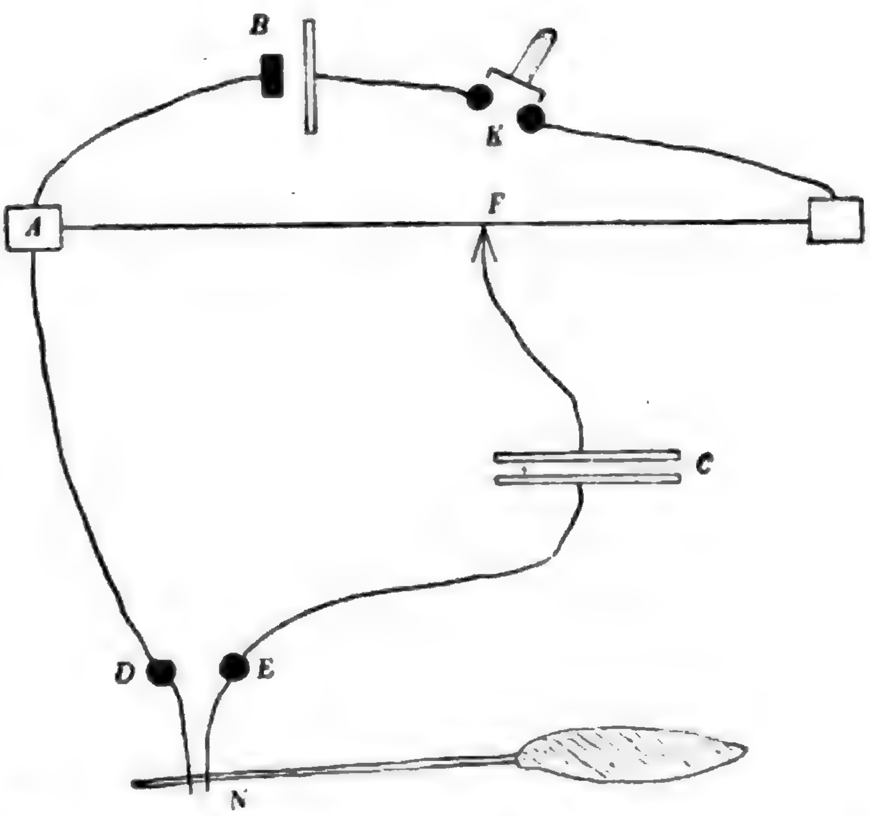

FIG. 102. ELECTRICAL CIRCUIT FOR USE WITH CONDENSER IN STIMULATING EXCITABLE TISSUES.

(Hermann, 1906.)

Although the condenser is the most perfect means of delivering accurately measured stimuli to a nerve, it requires somewhat complex apparatus when a rapid series of stimuli is required. For ordinary use, the induced currents produced in a coil of fine wire, by the make or break of a current in another coil of larger wire at an adjustable distance from it, are substituted. This arrangement, when fitted with an automatic interrupter, "Wagner's hammer," is known as "Du Bois Reymond's Coil," after its inventor. The currents induced by the make and break of a current in the primary coil differ in their time course, owing to the fact that the establishment of the current in the primary coil is retarded by self-induction, which is naturally absent at break, since the circuit is no longer complete. The break shock is therefore of a higher potential than the make shock.

Further details of the various methods of electrical excitation will be found in the article by Garten (1908). One or two facts may be mentioned here. The current used to excite must obviously enter the nerve at one electrode, and leave it at the other. It is always found that excitation takes place at the cathode when the current is established and, if it has lasted for some time, at the anode when it is broken. These facts can be made out best by the use of constant, unidirection currents, which can be kept closed as long as desired. Of course, when other than alternating currents are used, the electrodes must not be capable of polarisation. The construction of non-polarisable electrodes will be found in Garten's article (1908, pp. 333-339). A very convenient form is the modification of Ostwald's calomel electrode described by Noyons (1909), or that of Philippson (1912). No excitation occurs during the passage of a current as long as it remains

unaltered ; in fact, there must be a change of potential in order to excite, and this change must not be too gradual or it will not excite at all, nor too rapid, as the extremely rapid alternations of the Tesla currents, which are practically inactive in proportion to the energy which they contain. These currents are produced by induction from the rapid natural oscillatory discharge of a condenser, such as a Leyden jar, charged to a high potential by connection to a large induction coil or influence machine. A current of nearly half an ampere, sufficient to light an incandescent lamp in the same circuit, can be sent through the human body without exciting nerves therein. It appears, indeed, that what effects are produced by these so-called "*high-frequency*" currents are merely due to the heat into which they are converted in the tissues through which they flow.

A convenient method of application of currents of different time course is by the rheonome of von Fleischl, described in Garten's article (1908, p. 406). Keith Lucas (1907, 1) describes a simple form of rheonome, formed by an ebonite diaphragm with a hole, which is moved across another hole in a second shutter, which separates two compartments, each containing saturated solution of zinc sulphate, through which the current passes by means of a zinc electrode in each compartment.

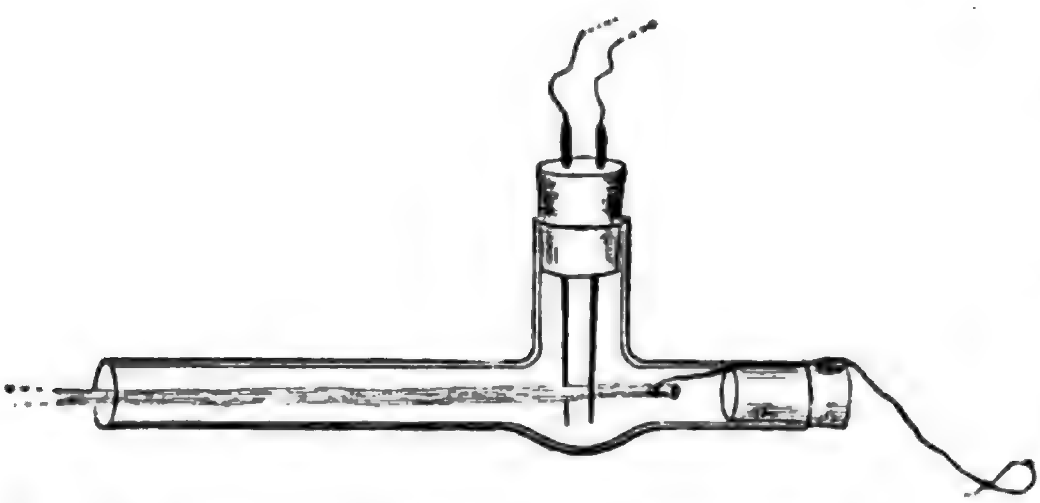

Fig. 103. Sherrington's electrodes for stimulating nerves.—The nerve is protected from drying, and the electrodes can be sewn up in the wound, or kept warm by a current of warmed saline run over them.

(Sherrington, 1909, 1.)

The use of alternating currents of sinusoidal form presents some advantages on account of the equality and regularity with which they can be made to stimulate. Currents with an approximately sine curve can be obtained by the rotation of a coil in a magnetic field, or vice versa, but to obtain mathematically correct curves requires very accurate apparatus. The alternating current supplied by central stations has nearly a sine curve and, when available, forms a convenient means of getting very regular, graduated, tetanising stimuli from an induction coil of the Du Bois Reymond type. It can be sent through the secondary coil and the electrodes connected to the primary, or vice versa. In the latter case, of course, a lamp resistance must intervene between the mains and the coil. The strength of the induced currents is varied by altering the distance between the coils

The various patterns of electrodes used for applying electrical stimulation to nerves will be found in Garten's article (1908, pp. 331-340). Two additional useful patterns may be mentioned here. The first is that used by Sherrington (1909, p. 382), especially for deep-lying nerves, and consists of a glass T-tube, into which the cut nerve is drawn, the current being applied by two platinum wires, one on each side of the nerve, passing through the side branch (see Fig. 103). They are also very good for superficial nerves, since they prevent drying and can be kept warm by a current of saline over the outside. The electrodes of Keith Lucas (1913, 2, and Fig. 104) are useful when it is necessary to excite nerves immersed in a saline solution, such as sea water or Ringer's solution, without the current spreading to neighbouring parts. The principle on which these electrodes are constructed is that the sectional area of the solution around the nerve is made to change very suddenly at the point where stimulation is desired and the current is made to pass by this course. Electrodes on the same principle, for the exact localisation of stimuli on the excised nerve muscle preparation, are described by the same investigator (1908, p. 114) and their degree of accuracy determined.

Nerves can also be excited by chemical means, as by crystals of salt or by

glycerol; the action is perhaps more strictly physical, since it seems to depend on the removal of water.

Mechanical methods, such as pinching, tapping, shaking, or snipping with scissors, are also effective stimuli, but obviously not capable of graduation. They produce more or less injury, so that they are used chiefly as a means of control when it is desired to exclude the possibility of a particular result obtained from a nerve being due to escape of electrical current to neighbouring parts. A simple apparatus for exciting nerves by dropping mercury upon them from different heights is that due to Schäfer (1901), which is capable, to a certain degree, of graduation in strength and rate of stimulus, and does not injure the nerve to an appreciable extent.

There are reasons for regarding all artificial modes of excitation as more or less unlike the natural one coming from the cell body of which the nerve fibre is a prolongation. It is possible, however, to exaggerate this difference; as we shall see later, the natural excitation is accompanied by waves of electrical disturbance, similar to those produced by artificial stimulation, and the optimal rate of incidence of energy, Waller's "characteristic," is probably very close to the natural one.

Nerves can be excited, then, by many and various forms of stimuli and, supposing that the nerve is in connection with some indicator, such as a muscle, different strengths of stimulation are found to produce different degrees of contraction.

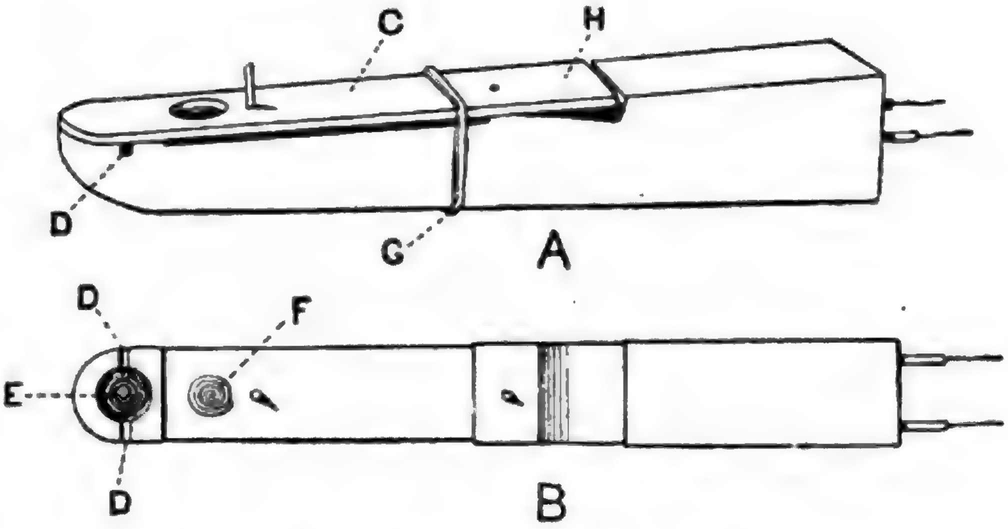

FIG. 104. ELECTRODES OF KEITH LUCAS.

For the prevention of escape of current when immersed in saline. The points of stimulation are at *D*, where the current density suddenly increases to a high value.

A, Side view.

C, Loose cover, kept in position by an elastic band, *G*; can be tipped up by pressing at *H*.

D, Channel for nerve.

B, View from above.

E and *F*, Spirals of fine platinum wire, connected with the wires at the right-hand end.

(Keith Lucas, 1913, 2.)

"*All or Nothing.*"—Now the most careful experiments (see especially those by Keith Lucas (1909)) have shown that the degrees of contraction of a muscle, that can be produced by varying the strength of the excitation of the nerve to it, are not as numerous as the degrees of strength of the exciting stimulus, but take place in a series of steps, which are no more numerous than the number of motor nerve fibres supplying the muscle. This fact obviously indicates that the varying degrees of contraction are due to differences in the number of muscle fibres in the state of contraction at one time, and that each fibre can only be excited to its maximal capacity or not at all. The fact had previously been established by Bowditch (1871, p. 687), for the heart muscle excited by stimuli applied directly to it. The possibility of its applying also to nerve itself was discussed by Gotch (1902, p. 407), who came to the conclusion that the magnitude of the electrical disturbance in nerve is conditioned far more, if not entirely, by the number of fibres excited, than by possible differences of intensity of the disturbance in individual fibres; but the actual proof was not given until the work of Adrian (1912). If we consider for a moment the case of a muscle being excited by shocks of varying intensity applied to its nerve, it will be clear that all the nerve fibres are not in exactly the same favourable position for receiving the stimulus, either

because of their more internal position and consequent short circuiting of the stimulus to a certain extent, or, possibly, owing to differences in their own state of excitability. This being so, a very weak stimulus would excite some and not others, thus causing contraction of a portion only of the fibres of the muscle. Although these latter may respond by a maximal contraction, the fact alone does not, however, prove that the impulse in the nerve fibre was a maximal one, since it might be only just sufficient to cause contraction.

A very ingenious method was devised by Adrian for the investigation of this and similar questions. All methods of experiment agree in showing that the disturbance, as it passes along the fibres of a normal nerve, suffers no diminution in intensity. If, on the contrary, the nerve is narcotised by the application of an anæsthetic, such as alcohol or morphine, a disturbance, started at one end, decreases in magnitude progressively as it travels along and, if the length or the degree of narcosis is sufficiently great, it is completely wiped out. Since the diminution in the disturbance is a regularly progressive one, it is clear that a smaller disturbance will be able to pass along a shorter distance of narcotised fibre without annihilation than one which was larger to start with. In practice, it is more convenient to vary the degree of narcosis, or period during which the anæsthetic is applied. The following description from Adrian's paper (p. 393) will assist the reader:—"The point to be decided is whether a disturbance which has passed through an area of decrement and entered normal tissue again is equal to or less than a disturbance which has been set up in normal tissue, peripheral to the area of decrement, and has consequently escaped any reduction. The following arrangement was adopted. Two muscle nerve preparations (sciatic gastrocnemius) are taken under exactly similar conditions. In one of these (Fig. 105, A) two equal lengths of nerve, d and d', are narcotised. These lengths are separated by a variable length of normal nerve and the conductance of a disturbance through one or both of them can be tested by stimulating electrodes I. and II., placed as shown in the figure. The other preparation is narcotised at the same rate over a length, D, which is equal to the two lengths, d and d', together. Conduction through D is tested by electrode III. Under these conditions the decrement suffered by the disturbance from II., in passing through d, will be equal to that suffered by the disturbance from III. in passing through the first (central) half of D. If the disturbance does not increase in size when it leaves d, it will enter d' in exactly the same condition as a disturbance which enters the peripheral half of D. In this case the depth of narcosis required to extinguish the disturbance altogether will be the same in both preparations, and stimuli at electrodes II. and III. will become ineffective at the same time. On the other hand, the disturbance from electrode I. will enter d' unreduced and therefore conduction from I. to the muscle will persist for some time after the failure at II.

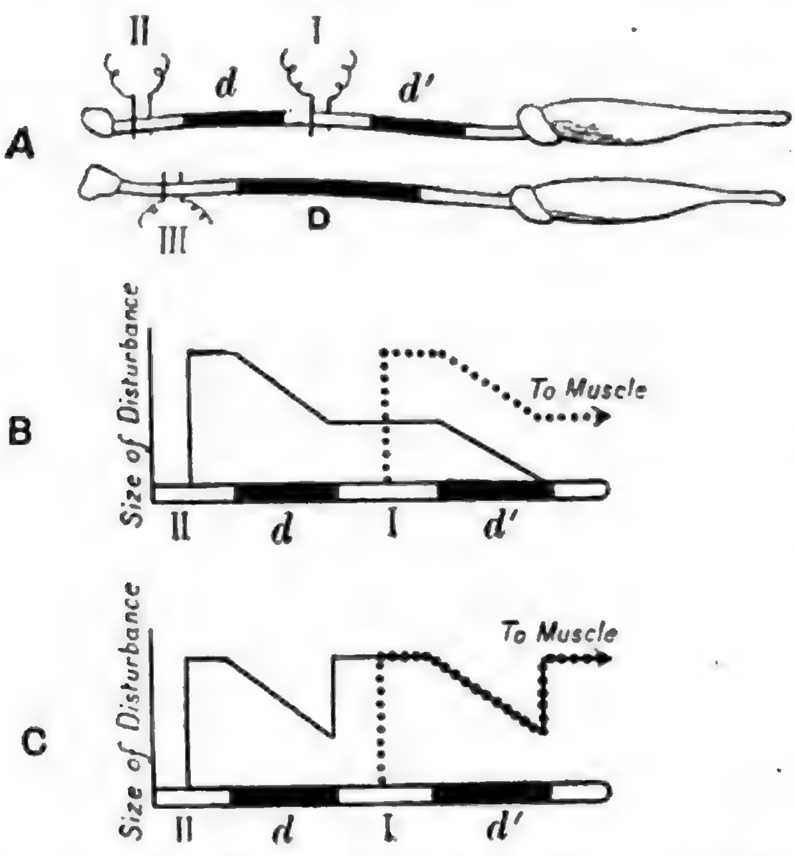

FIG. 105. ADRIAN'S DIAGRAM TO ILLUSTRATE THE RECOVERY OF A NERVE IMPULSE AFTER IT HAS PASSED THROUGH A REGION OF DECREMENT.

"Fig. 105, B may help to make this clearer. Ordinates are intended to represent the size of the disturbance at different points along the nerve during the

period when a stimulus at I. is effective as regards the muscle and a stimulus at II. is not. Thus the full line shows the disturbance starting at II., undergoing in *d* a decrement which does not lead to complete extinction, emerging into normal nerve between *d* and *d'*, where it persists at reduced magnitude, and then undergoing in *d'* a further decrement which does lead to extinction. The broken line shows the disturbance starting at I. undergoing in *d'* a decrement which does not lead to extinction and then passing on to the muscle in this reduced condition.

"Fig. 105, C shows what will happen if the disturbance recovers after leaving the region of decrement. In this case, the disturbance from II., when it travels through the normal tissue between *d* and *d'*, is fully equal to the disturbance which starts at I. (broken line). Thus stimuli at I. and II. will become ineffective at the same moment when the narcotic has acted for such a time that a full-sized disturbance is extinguished in the length *d* or *d'*.

"Consequently, the only data required for the solution of our problem are the times from the beginning of narcosis to the moments when stimuli from electrodes I., II., and III. cease to evoke a muscular contraction. If the stimulus fails first at III. and afterwards at I. and II. simultaneously, the disturbance must recover to its original size after leaving the area of decrement; if the stimulus fails first at II. and III. together and afterwards at I., the disturbance does not recover."

The actual experimental method used will be found in the original paper. Suffice it to say here that the results prove conclusively that a disturbance, after having been decreased by passing through a region of decrement, *recovers* its original magnitude when it re-enters a normal area. We may look upon the various magnitudes of the disturbance, as it emerges from regions of various degrees of narcosis and enters on the normal region, as being different degrees of intensity of a stimulus applied to the normal nerve. Experiment shows that the impulse then present in the normal region is the same in all cases and maximal, whatever its strength was after subjection to decrement.

Attention may be called to the method of measuring the strength of an impulse by the extent of decrement it can suffer without extinction. An important point in Adrian's work is that the strength was not measured by the magnitude of the electrical change alone, since the actual relationship of this change to the propagated disturbance itself is not, as yet, completely known.

Another way, in which the result is confirmed, is by applying stimuli of various strengths and allowing the impulses produced to pass through a narcotised region. It was found that the same degree of narcosis abolished all, so that they must have been of equal intensity.

By similar methods it was shown that, if the impulse is altered in magnitude by passing through a cooled area, it regains its original size on emerging into normal tissue.

Space does not permit discussion here of the results obtained by previous observers, which appeared to show a gradation of impulses in nerve fibres. Adrian (1914) has shown that they do not warrant the interpretation put upon them. There is one point, however, which should be referred to. It was thought at one time that a nerve might still be able to conduct a propagated disturbance through a narcotised area, when unable to respond to a stimulus applied directly to this area. The results of Adrian show that the phenomenon can be explained without this assumption, which, therefore, introduces an unnecessary complication. The phenomenon known as "Wedensky's inhibition" depends on the stage of diminished excitability immediately following the passage of an impulse which is known as the "refractory period" and will be discussed presently. With regard to the supposed distinction between conductivity and excitability, referred to above, the fact of the local excitatory change, which is antecedent to the setting up of a propagated disturbance and will be discussed below, should be kept in mind. This local state is not propagated and it does not appear improbable that the possibility of its occurrence might be prevented by the action of certain agents, although the nerve might still be able to conduct an impulse started elsewhere.

It appears to me that the results obtained by Adrian show quite clearly that there is no gradation of excitatory state in the normal condition, so that the fact must be accepted whatever consequences may follow from it. Its application to the phenomena in nerve centres and to heart muscle will be referred to later, but its

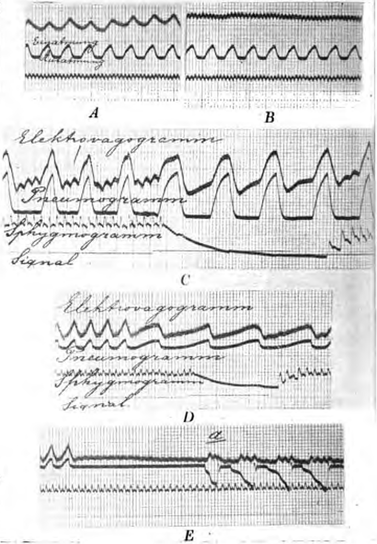

FIG. 106. ELECTRICAL CHANGES IN THE TRUNKS OF THE VAGUS AND DEPRESSOR NERVES IN NATURAL CONDITIONS. RECORDED BY THE STRING GALVANOMETER.

A, Vagus. Rabbit.

First curve from above—Electrical changes in the part of the cut vagus in connection with the lung.
Second curve—Respiratory movements. Inspiration upwards.
Third curve—Heart beats.
1 mm abscissæ = 0·2 second. 1 mm. ordinates = 3·5 microvolts.
Note that the electrical change is congruent with the degree of distension of the lungs, and that there is no change corresponding to the heart beats.

relation to the secreting glands may be mentioned here. If the varying degrees of secretory activity, to be obtained by gradation of the stimulus to the nerve, be due to maximal stimulation of a greater or less number of fibres, evidence should be obtained in microscopic appearances that some cells or alveoli are much more fatigued than others. If the figures on pp. 957 and 982 of Metzner's article (1907, 2) be referred to, it will be seen that this is actually the case.

A question cognate to the last, and of considerable importance, is whether electrical or other stimuli of different time course are able to produce *nerve disturbances of different kinds*. This would appear from Adrian's results to be improbable, but it has been found that the comparatively slowly rising current, to be obtained from a rheonome, caused an abnormally long twitch of the muscle to which the nerve was attached. Subsequent investigation with an instrument able to detect the existence of disturbances following one another at very brief intervals, showed that several successive impulses passed down the nerve in such cases. It appears that different nerve fibres are excited by the slowly rising current at different times after it begins to pass. Dr Keith Lucas, who gave me this information, also stated that he was unaware of any evidence to show that the nerve impulse is in any way modified by the nature of the stimulus. The different forms of electrical change in nerve must, therefore, be ascribed to a series of impulses, if it be supposed that they represent the process in a single fibre; but it seems more likely that a varying number of fibres are being excited in rotation. A case of this kind is shown in Fig. 106, D and E (Einthoven), where the electrical change in the vagus nerve, produced by inflation of the lungs, is seen to follow precisely the degree of inflation, and might be explained on the hypothesis that the receptive end organs in the lung tissue are of varying degrees of sensibility, so that all would be excited by a strong inflation, but fewer and fewer in proportion as the degree of stretching decreases.

That the result produced by the impulses travelling in a nerve depends on the way the fibres end, and not on any difference in the impulses themselves, is shown by Langley's experiments (1898) on the union of different nerves. When the central end of the vagus is joined to the peripheral end of the cervical sympathetic, and regeneration has taken place, stimulation of the vagus produces the same effects as that of the cervical sympathetic did previously. Moreover, reflexes produced by afferent impulses, which excite efferent fibres of the vagus in the normal state, instead of producing cardiac inhibition, cause contraction of the arterioles of the ear, together with the other effects of stimulation of the sympathetic. The central end of the lingual was joined to the peripheral end

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B, Depressor nerve. Rabbit.
First curve—Electrical change in the heart end of the cut depressor nerve.
Second curve—Respiratory movements.
Third curve—Heart beats.
1 mm. abscissæ = 0·2 second 1 mm. ordinates = 5 microvolts.
Note the electrical change with each heart beat, none with the respirations.

C, Vagus nerve. Dog.
First curve—Electrical changes in the thoracic end of the cut vagus.
Second curve—Respiratory movements.
Third curve—Blood pressure with heart beats.
Fourth line—Stimulation signal.
1 mm. abscissæ = 0·2 second. 1 mm. ordinate = 6·7 microvolts.
Note that both heart and lung produce electrical effects in the nerve, since the vagus trunk contains the depressor fibres.
At the rise of the signal the peripheral end of the vagus of the opposite side was stimulated. Respiration continues, with its electrical effect. The heart beat stops and, with it, the depressor waves cease.

D and *E*, Vagus. Dog under artificial respiration.
In *D* air is rhythmically blown into the lungs.
In *E*, after a pause, air is sucked out four times, commencing at *a*.
First curves—Electrical change in thoracic end of vagus.
Second curves—Movements of chest upwards means inflation.
Third curves—Blood pressure with heart beats.
Fourth line—Signal.
1 mm. abscissæ = 0·2 second. 1 mm. ordinates = 9 microvolts.
Note how the electrical change coincides with the curves of distension and continues during the whole period. It is not a momentary effect.
The direction of the electrical effect with suction is the same as that with distension, but of less magnitude.
The depressor effect of the heart beats is not well seen, owing to the decreased sensibility of the galvanometer. At the rise of the signal in *D*, the opposite vagus was stimulated.

(From curves kindly sent by Prof. Einthoven.

of the cervical sympathetic in another experiment. Feeding the animal, which normally produces reflex stimulation of vaso-dilators to the tongue and salivary glands, caused vaso-constrictor phenomena in the ear; direct stimulation of the lingual nerve produced vaso-constrictor effects instead of dilator effects. The conclusion seems inevitable that the excitation process in the nerve fibre is the same in all cases.

The question has important relations to the physiology of the sense-organs and is known as "Müller's law of the specific energies of the senses." It may be mentioned here that mechanical, chemical, or electrical stimulation of the chorda tympani nerve in the tympanic cavity cause equally sensation of taste. The sensation of light said to be caused by section of the optic nerve is not quite so certain, on account of the possibility of disturbance of the retina.

FIG. 107. REFLEX PRODUCTION OF MUSCLE TONE BY RHEONOME CURRENTS AND ITS INHIBITION.—Vasto-crureus of decerebrate cat.

At the top—time in seconds.
At the bottom—signal of stimulation.
Rise in the curve means contraction of the muscle.

The first period of stimulation is that of the central end of the popliteal nerve by weak rheonome currents. The *tonus increases*. The second period of stimulation is that of the same nerve, through the same electrodes, by a weak faradic current from an induction coil for one second only. *Inhibition* immediately results.

(Sherrington and Sowton, 1911, 1, Fig. 7.)

Differences in the state of excitation of a single nerve fibre, accordingly, can only be such as would be brought about by differences in the rate at which the separate stimuli follow one another.

We see now the difficulty of accepting the view of Babkin (1913) that the different effects of "secretory" and "trophic" nerves to the salivary glands are due to qualitative differences in the nature of the impulses passing to the gland cells along fibres of the same kind, that is, with similar peripheral and central connections.

That apparently qualitative differences in a reflex can be obtained by altering the character of the stimulus is shown also by the experiments of Sherrington and Sowton (1911, p. 439), where the form of the reflex contraction of the vasto-crureus muscle, produced by excitation of the central end of the popliteal nerve of the same side, varies in form according to the time course of the electrical stimulus used. For example, that produced by the rheonome current has the character of a long-maintained, steady contraction, without evidence of fatigue and with slow subsidence after the end of stimulation; in fact, it has the properties of the tonic contraction to be described in a later chapter (see Fig. 107). Of course, there are more varied possibilities in reflexes than in the phenomena to be obtained by direct excitation of the nerve observed; but it must be admitted that the explanation of reflex phenomena on the hypothesis of the "all or nothing" nature of the nerve impulse presents difficulties, as is pointed out by Graham Brown (1913). On the other hand, the tracings of reflex

contraction given by this experimenter show a number of steps of gradation which do not sufficiently exceed the possible number of motor fibres in the nerve to be satisfactory proof of the law failing to apply in this case.

As far as the number of separate nerve fibres in the motor nerves to the eye-muscles is concerned, it seems that they are fully sufficient to provide for all the degrees of contraction required. In the sixth cranial nerve, which supplies the external rectus muscle, the number of fibres is given by Zoth (1905) as 2,500 in man, and those in the nerve to the superior oblique muscle as 2,150. This fact, in itself, obviously suggests that different degrees of contraction are effected by changes in the *number* of muscle fibres stimulated. If there were a possibility of different degrees of activity in the same nerve—or muscle—fibre, a very much smaller number of individual fibres would be sufficient.

Refractory State.—It was first shown by Gotch and Burch (1899) that, if a stimulus is followed by a second one at an interval less than about 0·008 second, differing according to temperature, the second one does not give rise to a

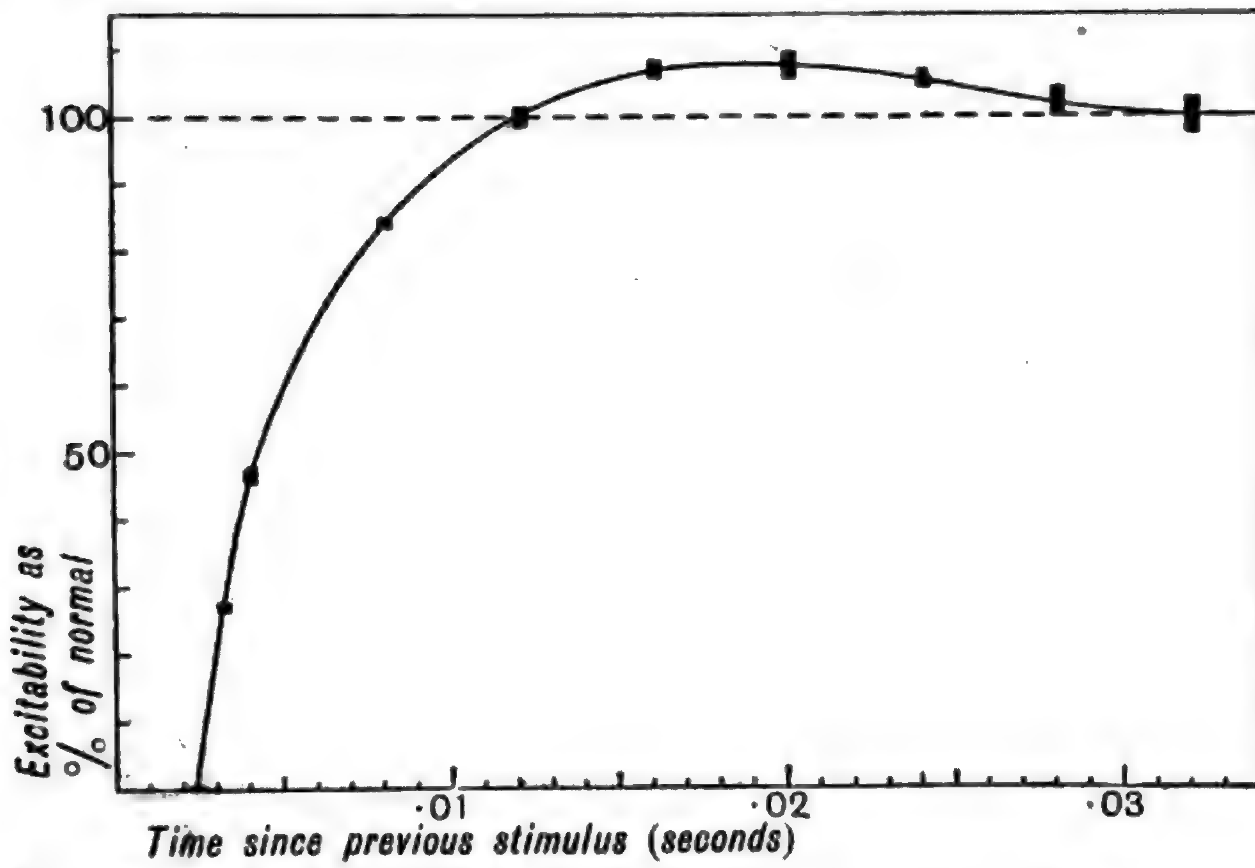


FIG. 108. CURVE OF RECOVERY OF EXCITABILITY OF NERVE AFTER A PREVIOUS STIMULUS.—The refractory state is, at first, absolute; excitability returns gradually, and becomes normal at about 0·012 second. It is followed by a brief stage of supernormal excitability.

(Adrian and Lucas, 1912, p. 114.)

propagated disturbance, as indicated by an electrical change. This means that the nerve is inexcitable immediately after a state of excitation. Further investigation of the state of the nerve during the period succeeding the passage of a disturbance was made by Adrian and Lucas (1912), by a method in which contraction of the attached muscle was used as indication of the disturbance in the nerve. Fig. 108 represents the percentage of normal excitability present at various intervals of time after the excitation, at a temperature of 14°·8 C. It will be seen that for 0·0025 second after a previous stimulus there is complete inexcitability to any strength of stimulus ("absolute" refractory period). From this time to about 0·012 second the excitability is lower than normal, gradually increasing; this is the period of "relative" refractory state, in which a stimulus stronger than normal is required to set up any propagated disturbance. Following this, until 0·028 second, there is a period during which the nerve shows increased excitability, to which reference will be made again presently.

During the relative refractory period, the disturbance set up by a stimulus which is just strong enough to excite is less than the normal one, as measured by its ability to traverse a narcotised region. In the normal nerve, as we have seen,

if a propagated disturbance is produced at all, it is a maximal one, and it is impossible to produce one of the smaller magnitude of those excited in the refractory state. The question arises, then, whether these smaller disturbances can be made greater by stronger stimuli. Adrian (1913) finds that this is impossible. So that the magnitude of the disturbance is conditioned only by the state of the nerve at the time.

The refractory state which follows a second effective stimulus, applied during the relative refractory state following a previous stimulus, is shorter than the normal one. The duration of the refractory state is, therefore, dependent on the magnitude of the disturbance.

The refractory state is not merely a local effect at the point of application of the stimulus, but is the same at any point in the nerve after the passage of the propagated disturbance (Bramwell and Lucas, 1911).

Keith Lucas (1911) shows further that the refractory state is associated with the propagated disturbance by the fact that a stimulus falling within the absolute refractory period of a previous one does not prolong that refractory state, and a third stimulus is effective at the same interval of time after the first, whether the second has been interpolated or not.

Fatigue.—If we regard fatigue as that result of activity by which a cell is less readily put into action again until a certain time for recovery has been allowed, it is clear that the refractory state itself is one of fatigue. Under ordinary conditions, however, the recovery is so rapid and complete that it is impossible to demonstrate that a nerve is less excitable at the end of a long period of activity than at the beginning. From certain experiments by Baeyer (1902), it appears that, in the absence of oxygen, signs of fatigue are to be detected; while the refractory period was found by Fröhlich (1904) to be prolonged to 0·1 second in the absence of oxygen. In view of the definite proof by A. V. Hill, that the heat evolved is so minute as to make it very doubtful whether there is any metabolism in the nerve fibre, it becomes necessary to consider for a moment what are the experimental facts with regard to the effect of oxygen. The experiments of Baeyer showed that a region of nerve, exposed to currents of nitrogen or hydrogen, failed to respond to induction shocks after some five hours' action. The excitability returned in oxygen. This behaviour is quite similar to that when a typical anæsthetic is used, so that it seems that the process may well be the same. Baeyer (1902, 2) did not obtain any evidence that the time required to produce the state of inexcitability was made shorter by continued stimulation of the nerve. Thörner (1909), however, found that continuous tetanic stimulation, in the absence of oxygen, caused an earlier appearance of the inexcitable condition; recovery took place, to a considerable extent, when the excitation ceased, *without* the necessity of the presence of oxygen.

It seems possible that the local effect of the current at the electrodes was not sufficiently excluded in these experiments. Polarisation is not easily prevented. Anyone who has excited the vagus nerve of the cat is familiar with the fact that the inhibitory effect on the heart rapidly disappears during stimulation and that reappearance occurs when the electrodes are moved to another spot on the nerve. The results of Baeyer may possibly have been due to traces of impurity in the gases used, although they were purified by the usual chemical methods. Exposure for five hours might enable an effect to be produced by traces which would be incapable of detection.

On the other hand, since we must suppose that nerve fibre is living, it is difficult to believe that it is absolutely devoid of respiratory activity. We know that it requires a certain minimal amount of energy to start a disturbance; but the fact that this disturbance in normal nerve is propagated without diminution, suggests a physical process, although it might be argued that energy is supplied to it as it travels. In the latter case, it is conceivable that the energy-giving material might require replacement by an oxidation process; but we are again met with the difficulty of the absence of heat production. A. V. Hill suggests that oxygen acts by keeping the machine in order, as it were, somewhat as oil in a motor does.

It must be confessed that this seems a rather unusual function for oxygen to perform, and it does not appear to me that the dependence of excitability on oxygen has been satisfactorily

demonstrated. It would be desirable to test the effect of a simple vacuum, although the experimental difficulties of exposing the nerve to the vacuum, while allowing access of oxygen to the muscle, seem insuperable; the use of the electrical change as indicator would be possible, though less satisfactory.

In any case, it will have been abundantly clear, from the various facts given in previous pages of this book, that the food requirements of cell *machinery* in general are extremely small; food is required to afford energy for the numerous physiological processes, and this is done by oxidation under the action of the cell mechanisms. An infinitesimal amount of oxygen may actually be necessary in such a process as that of conduction in nerve, where there is practically no energy change involved. We shall meet with some further facts bearing on the question presently.

Summation and Facilitation.—In the experiments of Adrian and Lucas (1912), from which the curve of Fig. 108 (p. 389) was constructed, we see evidence that the refractory period is succeeded by one of slightly increased excitability, in which a less strength of stimulus is required to excite a propagated disturbance. This phenomenon is met with in any part of the nerve after the passage of a disturbance. There is, however, another form of increased excitability, shown at the point of excitation only (Adrian and Lucas, 1912, pp. 69-72). The first effect of a stimulus at its place of application, is a process which, on Nernst's theory of excitation (see later, page 393), we should interpret as a concentration of ions against a semipermeable membrane. Now this happens even when the stimulus is too weak to set up a propagated disturbance. It is shown by the fact that a second stimulus, also inadequate by itself, following the first after about 0·0008 second, sets up a propagated disturbance. It is clear that the first stimulus has left behind it a change of some kind which persists for a measurable time, and is added on to that produced by the second stimulus when this is put in. The propagated disturbance, on the other hand, as we have seen, leaves behind it a stage of *diminished* excitability at this interval after a previous stimulus; so that there are evidently two factors involved in the excitatory process, one of which is confined to the point of application of the stimulus. The importance of this fact for the theory of excitation will be seen presently.

A narcotic, such as alcohol, does not prolong the time required for recovery, the refractory state, even at the stage in which the disturbance is conducted with considerable decrement and slowing of rate of conduction (Keith Lucas, 1913). This fact suggests that the recovery process is not of the nature of a chemical, oxidation process under the control of living protoplasm. In fact, it seems to exclude the view of the necessity of oxygen for recovery, as an oxidative process.

The Electrical Response.—We have seen that the disturbance in nerve is associated with a temporary state of negativity. The meaning of this will be discussed presently. It is held by some observers that the two processes are not necessarily connected, but Keith Lucas (1912, pp. 502-508) shows that none of their experimental results are free from objection and that there is no reason for doubting the identity of the two. On the other hand, he points out that more strict proof is desirable before definitely accepting the electrical change as a basis for a physico-chemical explanation of the excitatory process. In the case of muscle we shall find evidence that, although the excitatory process and the electrical response may be the same phenomenon, yet both these may be present without a contractile response, which is, so to speak, an additional process, whose conditions of appearance may be absent.

Macdonald (1902) gives good reasons for regarding the potential difference between cut end and longitudinal surface of nerve as due to the high concentration of inorganic salts in the axis cylinder, in connection with the presence of membranes impermeable to these. This potential difference was found to be abolished by a certain concentration of ions outside the nerve, 7 to 10 per cent. of potassium chloride being necessary. The salts of the nerve cannot be regarded as combined chemically and split off on excitation, but must be adsorbed on surfaces of colloids in the axis cylinder. In this way, as is pointed out, these salts are prevented from manifesting their great osmotic pressure. The difficulty, however, still exists, since ions, in order to give the necessary Helmholtz double layer, must be *free* and not adsorbed. The electrical state of nerve and muscle is often spoken

of as that of a concentration battery. As we shall see later (page 393 and Chapter XXII.) it is only in a modified sense that this statement can be made.

Under certain conditions it is possible to observe an electrical change in the opposite direction, after cessation of the stimulus, both after tetanising and after single stimuli (Garten, 1903, p. 59). This phenomenon, which was first noticed by Ewald Hering, was correlated by him with the restitution or assimilation process, by which the excited nerve returns to its original state. This view is in agreement with Hering's well-known theory of assimilation, which will be discussed under the head of "inhibition." But it cannot be said that we have, as yet, a satisfactory explanation of this positive electrical response. It may, perhaps, have some connection with the stage of increased excitability of Adrian and Lucas. According to Vészi (1912), however, the magnitude of an electrical response is *decreased* in the stage of positivity after a prolonged tetanic excitation, but it would be more to the point if the observations had been made on the actual propagated disturbance itself. Cremer (1909) suggests that nerve in the resting state may be in a condition of partial excitation or negativity, which disappears, of course, immediately after a disturbance, and would give rise to the appearance of a stage of less negativity, that is, of positivity, until the normal tonic state is re established. If the resting state is a balance of two opposite processes, as is likely, there is some justification for Cremer's view, although no other evidence has been brought forward in favour of the existence of such a tonic condition of partial excitation.

Certain support is given to the idea of the electro-positive response as representative of a restitution process by the experiments of Sochor (1911), who found that, in a current of nitrogen, this positive after-action is abolished much more rapidly than the negative excitatory change is. The result might be interpreted as showing the necessity of oxygen for restitution, but the fact that carbon dioxide was found to abolish the effect much more quickly than nitrogen does suggests rather narcotic action. As Garten remarks, granting the necessity of oxygen for the restoration process does not prove that it is an assimilation in the sense of Hering.

Rate of Conduction.—The fact that the nerve impulse takes time to traverse a nerve was first definitely shown by Helmholtz (1850) and had an important effect on views taken with regard to mental phenomena, since here was a nervous process capable of numerical expression.

The value obtained by Helmholtz for the frog was 29 m. per second. In man, the latest value, obtained by Piper (1912, p. 52), is 123 m. per second. This was obtained by the use of the string galvanometer and may be taken as a very accurate one.

All investigators agree that the rate is independent of the strength of the stimulus. Narcotics, such as alcohol, slow the rate of conduction (Keith Lucas, 1913).

The temperature coefficient as determined by the most accurate method, that of Keith Lucas (1908), is 1·79 for 10°. I have already pointed out (page 42) that it is not permissible to draw conclusions as to whether a process is chemical or physical from this value alone; one may say this much, that a simple chemical reaction with a temperature coefficient lower than 2, at ordinary temperatures, is extremely rare, if not unknown.

Changes in Permeability.—When a nerve is cut across and electrodes placed on the cut end and on the longitudinal surface, as in Macdonald's experiments referred to above, there is found to be a difference of electrical potential between these points, such that the cut end is negative to the normal surface. As we shall see in Chapter XXII., the only satisfactory way of explaining such electrical states is by the assumption of a membrane which is permeable to one of the ions into which an electrolyte inside the axis cylinder is dissociated, but not permeable to the oppositely charged fellow ion. We have, indeed, described such a case in that of Congo-red, separated from water by a parchment-paper membrane. A Helmholtz double layer is formed at the membrane or, as it is sometimes expressed, the membrane is "polarised," having anions on one side, cations on the other side. Suppose the membrane to become suddenly permeable to both ions, what will happen? Since the constraint preventing the two layers of ions from freely mixing is removed, the ordered arrangement of ions ceases to exist and, with it, the potential difference between the two sides of the membrane and the possibility of polarisation. Now this is precisely what happens when a nerve is put into a state of excitation. If the cut end is negative at rest and the other electrode on the longitudinal surface becomes negative when excited, as experiment shows, the potential difference is either greatly reduced or abolished,

according to the extent of the loss of impermeability at the excited spot. This is why the electrical response of nerve or muscle may be called, as by its discoverer, Du Bois Reymond, the "negative variation"; negative does not refer to the sign of the electrical response, but means diminution.

This manner of origin of the electrical response is sometimes described as a "concentration battery," but, if the description on pages 190-191 above be referred to, it will be seen that a concentration battery in the original sense requires electrodes of one of the elements of the dissociated salt. The electromotive force of the kind of battery with which we are here concerned is also a function of the relative concentration of the two solutions in the ion to which the membrane is permeable, and is expressed by the formula which Nernst worked out for the concentration battery proper, as will be shown in Chapter XXII.

If a potential difference is applied to such an arrangement, so that, for example, the anode is on that side of the membrane where are the cations, to which we will suppose the membrane to be impermeable, and the cathode on the opposite side, it will be clear that no current will flow, since no cations can travel to the cathode to be discharged there. The membrane is said to be polarised. If, however, the membrane becomes completely permeable, the current can pass freely, and the polarisation ceases. This change was shown by Hermann (1879, pp. 165-167) to occur in the excitation of nerve; it becomes less polarisable, as it might be expressed.

The fact may also be stated in the form that the excitatory change is increased at the anode, diminished at the cathode. Verzár (1912) has obtained results which show that this diminished polarisability lasts considerably longer than the electrical excitatory change proper, although in a diminished degree.

Further direct evidence of increased permeability of the membrane in excitation will be found in the case of muscle below.

Confirmation of this view of the source of the electromotive force of nerve is to be found in the experiments of Macdonald (1900) on the magnitude of the "demarcation current," when immersed in solutions of electrolytes. This "demarcation current" or potential difference between cut end and normal surface follows the Nernst formula for concentration batteries, as we have seen that the membrane process of the hypothesis in question does.

It must be confessed that it is difficult to make out at present which of the two changes referred to is the cause of the other, or whether they are different expressions of the same phenomenon. The loss of impermeability may be the cause of the disappearance of polarisation, or the disappearance of polarisation, as in excitation by an electrical current, may affect the membrane, which must be colloidal in nature, in such a way as to make it permeable. But it is not easy to see how *mechanical* stimuli can directly affect polarisation.

When the excitability of nerve is spoken of as a *colloidal phenomenon*, what is to be understood is that the membranes of which we have spoken are of complex colloidal structure and, as such, sensitive to electrolytes, etc. Hoeber (1910) has shown that electrolytes, in their action on nerve, follow the Hofmeister series, a characteristic of their action on lyophile colloids, as we have seen. Loewe (1913), also, shows how the action of narcotics is to be explained as a decrease of the possibility of the membrane becoming permeable on excitation. This decrease is due to adsorption of the narcotic by the preponderant lipoid constituents of the membrane, which are thus changed from lyophile to lyophobe colloids.

The Nernst Theory of Excitation.—Nernst (1899), considering the reasons why very rapidly alternating currents do not excite nerves, was led to the view that the process of excitation by an alternating electrical current is essentially connected with the production at some membrane of a certain minimal concentration of ions to which the membrane is impermeable. If the time during which the current passes in any one direction is too short, the opposite current will carry back these ions before they have had time to reach the effective concentration. This view leads to the simple law that single currents of variable duration will be of the same just effective strength if the product of their strength and the square root of their duration is constant. This follows from the mathematical expression for diffusion. Now, experimentally, this simple relation is found to hold only in a limited region of very short durations of current flow. In fact, Nernst himself regards it only as a first approximation and suggests factors that have to be taken into consideration in a law of wider application. Some of these factors have been considered by A. V. Hill (1910) and modified formulæ put forward.

The factors in question may be discussed briefly here. It may be pointed out that, from the standpoint of general physiology, the value of formulæ of the kind in question is not so much that of being able to express the relation between the exciting power of an electrical stimulus and its physical properties, but the light that they throw on the nature of the excitatory process itself.

The first point is that, in Nernst's treatment of the problem, only one membrane is taken account of. But it is clear that there may be another membrane at no great distance from the one under consideration, which will make a considerable difference in the diffusion of the ions, since the ions of opposite sign will be concentrated there. By the introduction of this conception, Hill deduces a formula which was found by Keith Lucas (1910) to satisfy experimental data when currents of long duration are used. The effect of the proximity of the membranes in its tendency to cause the equalisation of concentration by diffusion, owing to the rapid fall of concentration in a short distance, would naturally not come into play in very short periods of closure of the current.

A second point, which was suggested by Nernst in order to account for the fact that, if a current is allowed to rise in strength at a rate less than a certain critical value, it does not excite at all, is that there is reason to suppose that the separation of ions brought about by the current is accompanied by a slow, independent, automatic process, by which the ions are taken out of the sphere of action in some way before they have attained sufficient concentration to excite. The precise manner in which this happens is not clear, but it is probably a reversible process of the nature of adsorption.

Hill gives (1910, p. 208) as an illustration a tube of a mixture of oxygen and hydrogen gases. Suppose that this is heated at one end to a temperature at which explosion occurs. This corresponds to an effective stimulus setting up a propagated disturbance. But, if we heat very gradually, not allowing the temperature to rise to the explosion point, the gases combine slowly without explosion, and, if the heating is continued for a sufficiently long time, there will be a very small tension of the gases left uncombined, and no explosion will result even when the temperature arrives at the degree usually sufficient.

According to Hill, the experimental results available at present are not of such a form as to enable his formula to be applied to cases of exciting currents slowly rising in strength.

Although the complete derivation of the formula is beyond the space that can be given here, it may be of interest to enumerate the factors of which it consists. In its simplest form it is:—

$$i = \frac{\lambda}{1 - \mu\theta^t}$$

where i and t are the variables, i being the smallest current that will excite when of the duration t. λ, μ and θ are constants, whose precise form and significance will be found in the original paper and in that by Keith Lucas (1910, p. 234). It must suffice to say that each of these constants is compounded of other constants to which a definite meaning can be attached. They are:—

- a, the distance between the membranes.
- b, the distance from the membrane at which the concentration changes are being considered.
- k, the diffusion constant of the ion concerned.
- r, the number of ions, each carrying a given quantity of electricity.
- C, a constant expressing the rate of "recombination" of the ions in the manner referred to above; or, as Lucas prefers to put it, the ease with which the propagated disturbance is set up in a particular condition.

Lucas shows further (1910) how the various constants are affected by certain changes of condition, such as temperature and presence of calcium, and the part played by each in the process of excitation. We note especially the changes in C and in k. Now $\frac{k}{a^2}$ is the diffusion time of the ions concerned in the process, and the constant θ of the simplified equation is defined by Hill as

$$\theta = e^{-\frac{k\pi^2}{a^2}}$$

or

$$\log \theta = -\frac{k\pi^2}{a^2}$$

so that a convenient measure of $\frac{k}{a^2}$ is log θ. Lucas calculates the value of log θ for various excitable tissues from his own experimental data, to which reference will be made presently. It may be noted that, in all probability, this quantity is essentially the same thing as Waller's "characteristic." In fact, any considerable alteration in the shape of the curve correlating excitation with stimulus is due to changes in θ, since λ and μ are not so readily affected, as may be seen by the consideration of what they mean.

λ is the smallest current that will excite at all, however long it be continued. If t becomes very large, $1-\mu\theta^t$ becomes unity, and i is equal to λ.

μ refers only to the distance from the membrane at which the change of concentration is being considered, and will not be liable to important changes.

C enters into λ but not into μ or θ.

I fear that this necessarily brief account gives but an imperfect view of this important work; the original papers of Hill and Lucas should be consulted.

There is another point to which a little attention must be given. The fact, that on closing a current through a nerve, the excitation wave starts from the cathode shows that the cations are the important agents. How then is the fact of excitation at the anode, which occurs on breaking the circuit, to be explained? It is pointed out by Keith Lucas (1912, p. 519) that "the one feature which is common to the cathode when the current is made, and the anode when the current has just ceased to flow, is an increase of the concentration of cations above the value which occurred at each of these points immediately before." At the anode, however, the concentration of cations only rises to its normal level by diffusion, after having been decreased. Nernst and A. V. Hill give what is essentially the same explanation on the ground of the "combination" of ions with some substance in the nerve. During the passage of the current, the diminished concentration of cations at the anode results in a different equilibrium in the reversible "compound," or adsorption, between the ions and the assumed substance. When the current ceases to flow, there is a sudden concentration of cations in the system in excess of that with which it was previously in equilibrium; a condition which is the same as that at the cathode when the current is first established. Thus the excitation at the anode and the failure of slowly rising currents to excite appear to depend on the same conditions. It will be clear that more experimental work is required before the question can be decided.

A word is perhaps necessary as to the position of the membranes about which we have been speaking. There is no evidence of the existence of transverse membranes and, in fact, their assumption would raise considerable difficulties. It seems most likely that it is the cell membrane covering the axis cylinder that is concerned. This axis cylinder, as we shall see, is a part of a long cell, the "neurone," which includes the cell body with its nucleus, etc. Bernstein (1902), indeed, put forward the view that this surface membrane is the structure responsible for the electrical phenomena of nerve and muscle.

As already pointed out in Chapter III., it is not necessary to suppose that this membrane is in the form of a distinct film, or separate phase, which could be picked off. Being formed by condensation of constituents present in either or both of the two phases, cell protoplasm and surrounding liquid, of which it is the contact surface, it may be looked upon as belonging, in a certain sense, to both. We may notice that Mines (1912, p. 230) comes to similar conclusions in explanation of the results of his work on the effect of ions on the electrical charge of surfaces.

Our consideration of Nernst's theory of excitation may be best concluded with the words used by Keith Lucas (1912, p. 524): "It is not a complete theory, ready for acceptance, but it is an indispensable guide to the strengthening of our experimental data, and so to the ultimate elaboration of a hypothesis, which shall be free from those difficulties which are at present so obvious." The nature of the local excitatory process is especially in need of further investigation. It seems, however, from what has already been done, that the final solution will be on the lines of that proposed by Nernst.

Structure of Nerves.—The fact is familiar that some nerve fibres are encased in a sheath of considerable thickness. This consists chiefly of lecithin and similar lipoids, containing the radicles of unsaturated fatty acids and, therefore, staining with osmic acid. The function of this "*medullary sheath*" is problematical. It is obviously not necessary as an insulator, since many nerve fibres are devoid of it,

and we can see, by taking such a case as that of the superior cervical ganglion, that the non-medullated fibres are isolated from each other. Medullated fibres leave the spinal cord and proceed to this ganglion, where they form junctions, "synapses," with another set of neurones, whose fibres are not medullated. In this ganglion, therefore, non-medullated fibres of various functions are mixed together, but isolated functionally. These various kinds of fibres are vaso-constrictors, dilators for the iris and nerves to the different secretory glands.

Even if, as some state, the medullary sheath is formed by cells independent of the neurone itself, it must have an intimate connection with it, since, on cutting the axis cylinder free from the nucleated cell body to which it belongs, the medullary sheath undergoes degeneration along with the axis cylinder. When nerve fibres regenerate by growing out again from the cells, the remains of the old sheaths seem to act as guides for the new fibres, which grow down into them.

It has been suggested that this myelin sheath may serve as a source of nutrition to the fibre. It is very doubtful, as we have seen, whether there is any metabolism in the nerve fibre to require food.

After treatment with fixing reagents, the contents of the axis cylinders appear as a number of filaments, "*neuro-fibrils*," as they have been called. There is no actual proof of their presence in the living state, and they are, in all probability, produced by the action of the reagents used. Mott (1912) finds no indication of their presence in the living nerve cell.

On the contrary, Carlson (1911) has brought forward evidence to show that the axis cylinder has the properties of a liquid, just as protoplasm in general. Certain animals, such as the slug, exhibit great changes in their length; the nerve fibres must be stretched in the long form of the slug, since they are not folded up in the shortened condition of the animal. Now, Carlson finds that, if the pedal nerves are excited close to the pedal ganglia with the animal artificially stretched and again when unstretched, the time which elapses before the foot muscle contracts is greater in the former case. It is important to note that the degree of stretching was not such as to affect the excitability of the nerve, which was tested by using submaximal stimuli, and the same height of contraction found in both cases. If the nerve is stretched too much the muscle enters into contraction, and also the extent of the stretching was about that of the normal crawling movements. What are we to conclude from the result obtained? It is evident that the nerve itself was actually stretched and not merely uncoiled, since the latter would have had no influence on the time of conduction. It is difficult to understand how any substance but one having the characters of a liquid could be increased in length without showing any result beyond an increase in time of conduction proportional to the increase in length. Carlson, in fact, shows that the *rate* of conduction is unaltered. Since increase in length implies decrease in diameter, the result indicates that the rate of conduction is independent of the sectional area of the axis cylinder.

Ehrenberg showed that nerve fibres are doubly refracting, and the fact was confirmed by Ambronn and Held. Göthlin (1913) finds that the statement applies to both the medullary sheath and the axis cylinder. That of the latter is of the kind shown by proteins and is slight. That of the former is similar to that of liquid crystals of phospho-lipines. These crystals are arranged in a radial manner, so that they present themselves in a different way to a beam of polarised light, thus showing a cross, when a fibre is observed in section. Fibres in invertebrates, apparently non-medullated, show a sheath to polarised light.

The Nature of the Nerve Impulse.—It may be useful to collect together the evidence obtained so far on this question.

That it is a reversible, physico-chemical process, not associated with loss of material on account of metabolic reactions, is indicated by the following facts:—

Incapability of fatigue under normal conditions.

Absence of formation of heat.

Absence of decrement in wave.

Low temperature coefficient of rate of conduction.

No conclusive evidence of metabolism of any kind.

On the other hand, the existence of fatigue in the absence of oxygen points to

a minimal consumption of material for energy purposes, although it seems to me that the evidence on this question is not so decisive as it should be, and that further investigation is necessary before it can be interpreted in the sense mentioned.

A distinction must be made, as we saw, between the local process at the spot excited, a process which is not propagated, and the propagated disturbance set up when the former exceeds a certain magnitude. A stimulus must, therefore, possess a certain minimal amount of energy in order to excite, and it seems that this is required to effect the local change. No further supply of energy appears to be necessary in the progress of the wave along the nerve fibre. In the natural connection of the nerves with their cell bodies, the energy required to start the initial process is, no doubt, supplied by the cell body, in which oxidation processes of a recognisable degree are known to occur.

The conditions in the nerve fibre which are apparently concerned in the process are the polarisable membranes, of colloidal structure, and electrolytes, present in the complex, liquid, colloidal system of the axis cylinder.

The account given by Macdonald (1905, pp. 331-350), and Lillie's paper (1916) on the mode of propagation in nerve, will be found very instructive.

Although the evidence seems to preponderate on the side of the physico-chemical theory, it must not be forgotten that certain phenomena are not easy to explain on this view. Keith Lucas has pointed out that the propagation of disturbances along wires or similar channels may be of two kinds: (1) Like that of sound waves, or the passage of an electric potential difference along a condenser system, such as a submarine cable; this is purely physical, and does not involve production of energy as it travels. (2) A chemical system, such as a train of gunpowder. In this case, energy is evolved as the disturbance is transferred from one point to another, and products of change are given off. Now, Adrian's results, showing that a disturbance recovers its magnitude in a normal region, after having been reduced in a narcotised one, suggest a process more like the second one. If a sound wave, for example, is reduced in magnitude by passing through a region in which sound is conducted badly, say cotton wool, the energy of its vibration is diminished, and there is nothing to increase it again when it returns to a medium conducting well. On the other hand, if the train of gunpowder be made very narrow at one part, so that the energy of the disturbance is much less as it traverses this part, the original energy is regained when the dimensions of the train become similar to what they were originally. As long as the disturbance passes through the narrowed part at all, the original magnitude is regained in the normal part. Of course, the process in nerve cannot be regarded as being so simple as this; there are evidently physico-chemical processes connected with the movement of ions and the presence of semi-permeable membranes, and we are at present in the dark as to the way a reaction associated with the giving off of chemical energy comes into relation with the former. It is evident, however, that if, in excitation, the membrane ceases to be semipermeable, so that the internal electrolytes diffuse out, some supply of energy may be necessary in order to restore the original state of the system (see Lucas, 1917, pp. 23-27).

THE PROCESS OF EXCITATION IN MUSCLE

The chief function of the tissue known as muscular is that of producing movements by shortening or change of tension. This aspect of its activity will be considered in the next chapter.

Regarded as excitable tissues, muscles show very much the same characteristics as nerves. They can be set into activity by the direct application of a stimulus, which sets up a wave of excitation, which travels at a slower rate than that in nerve.

The *Refractory Period* is longer than in nerve, and can be particularly well seen in the muscle of the heart.

Muscle shows the "*all or none*" phenomenon, as shown especially by the work of Keith Lucas (1909) and that of Pratt and Eisenberger (1919).

There is an *electrical change* similar to that in nerve.

In the state of excitation there is evidence of *increased permeability* of the muscle cell, evidence of a more direct nature than in the case of nerve. The observations of Lillie (1911) on the larva of *Arenicola* have been already referred to (pages 138 and 139). Some experiments in which substances such as bile salts, saponin, and sodium oleate, which are known to make the cell membrane permeable, were found to cause quick, vigorous twitches of frog's muscle, are reported in the same paper.

The same fact is shown by the increased electrical conductivity of striated muscle in a state of excitation, as in the experiments of M'Clendon (1912, 2). This means that the membrane becomes permeable to ions to which it was impermeable while unexcited. The state of polarisation, in other words, ceases to exist. In general, the remarks made above with respect to the similar change in nerve apply also to muscle.

A further fact which indicates increased permeability is that found by Siebeck (1913). Potassium chloride enters more rapidly into excited muscles than into resting ones.

If the change of semipermeability into permeability is essential to the act of excitation, it will readily be seen that, while this state lasts, there will be a "refractory period." There is evidence also that the duration of the electrical change coincides very closely with that of the refractory state, as would be expected to be the case if this electrical change were due to the disappearance of the state of impermeability to the ions of one sign of charge, with the consequent depolarisation at the membrane.

In muscle, however, we find an additional factor, that of *contraction*, by which energy is given out. Along with this, phenomena are shown by muscle which nerve does not show. These will be treated of more fully in the next chapter; but there are four properties of muscle which are connected with this factor that should be mentioned here. They are latent period, metabolism, heat production, and fatigue.

Latent Period.—The state of excitation indicated by the electrical change, commences at such a short interval after the application of a stimulus, that it is difficult to be certain that they are not simultaneous. There is, on the contrary, an interval which can easily be measured before the state of contraction begins. If the electrical change were unknown, it would appear that nothing was taking place in the latent period before contraction.

It appears, then, that there is, in muscle, an extra mechanism superadded to the simple excitation process, namely, that giving rise to the contractile effect.

There is direct evidence that the propagated disturbance, with its electrical change, can continue in muscle which has been treated in such a manner as to show no trace of contraction. Härtl's experiments are the most convincing. If a part of a muscle be immersed in distilled water, it will be found to be incapable of contraction when stimulated, although this waterlogged part will still conduct a disturbance to the normal part. Noyons (1908 and 1910) has shown that certain drugs will abolish the beats of the heart of the frog and tortoise, while leaving the electrical change still strong. And Mines (1912, 2) has shown that skeletal muscles of the ray, treated with a dilute solution of ether, completely lose their power of contractile response to strong electrical stimuli, while retaining that to acid, alkali, or potassium salts. Presumably, the loss to electric stimuli was due to failure of conduction of a propagated disturbance. In the same paper, Mines refers to his observations that the conduction of the excitation process in heart muscle is arrested by trivalent cations, whereas the contractile process is not so affected (see Fig. 172 below).

Owing to the fact that this contractile process is one attended with the performance of external work, we find phenomena not present in nerve—consumption of oxygen, giving off carbon dioxide, production of heat, fatigue, and so on, all of which belong to the subject of the next chapter.

In order, however, to throw light on the process of inhibition, it is necessary to make use of the states of contraction and relaxation as indications of what has happened.

We have already seen that there is a distinction between striated, skeletal,

"voluntary" muscles, with their rapid contraction, and the smooth, "involuntary" muscles of the viscera and blood vessels, with their slow rate of contraction. The latter are, in their natural, unstimulated condition, in a state of partial contraction, so that two sets of nerves are required, one set to increase the activity, which may therefore be called "excitatory," the other set to decrease it, "inhibitory" nerves. The voluntary, skeletal muscles are, if unstimulated, completely at rest. They are supplied with one set of nerves only, those causing excitation, the other being needless. If continued tonic contraction is required, it must be kept up by continued innervation from the nerve centres; so that, to inhibit this state of contraction, influences must be brought to bear on the nerve centres themselves to stop their activity. It is unnecessary to remark that the excitatory and inhibitory centres of the smooth muscles are also liable to similar exciting and inhibiting influences. There are, then, at least two kinds of inhibition, one exercised on muscle itself directly, the other on nerve cells, when these are in a state of activity. Again, the inhibitory nerves of smooth muscle arise from centres in the nervous system, and these centres, if in a state of activity, can be inhibited by the play upon them of nerve impulses from other sources. We have thus, in Sherrington's phrase, an "inhibition of inhibition," that is, a central inhibition of nerve activity which was producing inhibition in peripheral organs. We shall find evidence of the actual occurrence of this phenomenon in the case of vasomotor reflexes.

OTHER EXCITABLE SUBSTANCES

The two excitable substances already discussed, muscle and nerve, are not the only members of the class. It might be supposed that a nerve acting on a muscle merely formed some kind of direct connection therewith, but a simple experiment shows that there is something between them, itself an excitable substance.

Let us take two nerve-muscle preparations and arrange the nerves of both on similar electrodes in series in the same circuit, so that they can be excited with the same strength of stimulus. On the one nerve, between the seat of excitation and the muscle, we place a pair of non-polarisable electrodes, through which we send a galvanic current of sufficient strength to block the nerve impulses on this side. Both nerves are thus excited, one muscle only. After a time, the muscle becomes fatigued and ceases to respond. At this moment, the galvanic current causing the block is cut off; the muscle on this side goes into tetanus. In this way, it is seen that the fatigue was not localised in the nerve trunk. The next step is to apply electrodes directly to the muscle which had ceased contracting; it is found to be able to respond vigorously. So that the *seat of the fatigue* is not in the actual contractile substance of the muscle. The unavoidable conclusion is that there is some intermediate substance, more easily fatigued than either nerve or muscle.

The action of the arrow poison, *curare*, affords similar evidence. If the nerve only is immersed in a solution of this drug, it is not paralysed. If the muscle is immersed, excitation of the nerve has no effect upon it; but it is not because the muscle itself is paralysed, since placing the electrodes on it produces contraction.

Under the microscope, there is to be seen, where the nerve enters the muscle fibre, or rather comes into connection with it, what appears to be a specialised structure, the "*end-plate*"; but that this is not the substance for which we are seeking is clearly shown in several ways. Adrenaline, as we shall see in more detail in Chapter XXIV., is a secretory product of the suprarenal bodies and has the property of exciting organs supplied by sympathetic nerves, and in precisely the same way as excitation of these nerves themselves. When, therefore, it is applied to arteries innervated by vaso-constrictor nerves from the sympathetic, these arteries contract. On the other hand, if applied to arteries not supplied by sympathetic vaso-constrictor nerves, no contraction results. It does not, accordingly, produce its effect by direct action on the muscle cells. If the nerves are cut and allowed to degenerate, the constrictor effect of adrenaline is undiminished. Now there is every reason to believe that the visible nerve endings in muscle degenerate with the nerve fibre.

Langley (1906, p. 179) finds that the nerve endings on the sartorius muscle of the frog disappear in six weeks after section of the nerve to the muscle. There is, moreover, no

histological evidence of any difference between the fibres in the trunk of the nerve and their endings on the muscle. It is evident that the intermediate substance, on which adrenaline acts, lies on the muscle side of the place of entry of the nerve fibre. Elliott speaks of it as the "myo-neural" junction (1905, p. 436).

Further light is thrown on the question by Langley's work on the antagonism between *nicotine* and curare (1906). Nicotine, in fairly large doses, acts like curare in preventing excitation of a motor nerve from reaching the contractile substance of the muscle. In the fowl, 10 to 15 mg. suffices. The first effect of the injection is to cause contraction of the muscles; but the remarkable thing is, that, after a dose which paralyses the nerve action, direct application of the drug to the muscle itself still causes tonic contraction. Further, this effect is abolished by curare. There is, in fact, a quantitative antagonism between the two substances. If nicotine be given after a dose of curare sufficient to paralyse the effect of nerve stimulation, a tonic contraction is caused. Repeated doses of nicotine finally paralyse the structures at first excited by it, although the muscle is still excitable to electrical stimulation; this is a further proof of some intermediate substance. As we have seen, curare acts on something on the muscle side of the nerve ending and nicotine must also act on the same substance. This constituent of the neuro-muscular system, which is not the contractile substance of the muscle nor the excitable substance of the nerve, is called by Langley the "*receptive substance*." It receives the stimulus from the nerve and transmits it to the contractile mechanism of the muscle.

We may now consider the evidence brought by Keith Lucas from a different point of view. In making experiments on the excitation of muscles with condenser discharges to find the constant called by Waller the "characteristic," Lucas (1906, 1) found that there were two distinct optimal stimuli, in one of which the rate of incidence is represented by 37 to 63 and in another of which it is represented by 1,780 to 19,300. After moderate doses of curare, these are both left present, although that with the higher optimal rate of incidence of energy shows signs of abolition, which is complete with large doses. It appears that we have to do with something analogous to Langley's receptive substance or Elliott's myo-neural junction.

In further investigation, Lucas (1906, 2) found that the end of the sartorius muscle which is free from nerves shows only one optimum, represented by 20 to 36. The trunk of the sciatic nerve also has an optimal rate represented by 41 to 233 only. Muscle fibre, free from nerve endings, has, therefore, an excitable substance (α) of low optimal rate. The nerve trunk has one (γ) of slightly higher value. In the middle of the sartorius there are at least two, detectable by the use of the condenser; there is the muscle itself as above (α) and another (β) of an extremely high optimal stimulus, on the muscle side of the curare block. In later work (1906, 3 and 1907, 1) it was found better to use currents of varying strengths and durations in place of the condenser discharges, and curves were drawn correlating the current strength just sufficient to excite with the current duration. In this way, the three substances above mentioned were found in the middle region of the sartorius. The current strength used was, in all experiments, such that its necessary duration never exceeded 0·02 second.

It was pointed out above (page 394) that the logarithm of the constant θ of Hill's modified Nernst formula of excitation is a function of $\frac{k}{a^2}$, which is itself a measure of the rate at which the diffusion of the ions concerned in excitation takes place. It is natural to suppose that the rate of incidence of energy in the optimal stimulus will be related to this factor, and Keith Lucas calculates (1910, p. 245) the values of log θ for various excitable substances as follows:—

Substance β of sartorius	2
Motor nerve fibres	0·3
Muscle fibre of sartorius	0·07
Ventricular muscle of heart	0·0005

These are arranged in order of rate of diffusion of ions. Compare these with the

values of the current durations at which the current strength reaches its smallest values, that is, the optimal rate of incidence of stimulus: we have:—

Substance β	0·0009 second
Nerve fibre	0·003 „
Muscle fibre of sartorius	0·02 „
Ventricular muscle	2·00 seconds

Some interesting conclusions are drawn by Keith Lucas from these figures. If the ions concerned in the excitatory process were the simple inorganic ones, $K^{\cdot}$, $Ca^{\cdot\cdot}$, Cl', etc., the variations in rate could not exceed 10 to 1, whereas between substance β and ventricular muscle there is a ratio of 4,000 to 1. The temperature coefficient is also higher than that for a simple ionic velocity. Similarly, when the calcium of the Ringer's solution is replaced by an isotonic amount of sodium, the value representing the rate of movement of the ions in excitation (k) decreases ten times, whereas the ratio of the velocities of $Na^{\cdot}$ to $Ca^{\cdot\cdot}$ is as 44 to 53 only. There is evidently some factor not as yet completely accounted for, and the question requires further investigation.

Lapicque, who has worked out the theory of electrical excitation and come to certain conclusions similar to those of Lucas, but without arriving at a mathematical form of his hypothesis, has made a hydrodynamic model (1909) on which many of the facts can be demonstrated; the movement of ions is imitated by that of water. Lapicque has introduced a constant which he calls "chronaxie," relating to the rate of movement of the ions concerned in excitation, which constant is of similar significance to Waller's "characteristic" and the logarithm of the constant θ of Hill's modified Nernst formula. Lapicque and Legendre (1913) find that there is a relationship between this rate of movement and the diameter of the various nerve fibres in the same animal. The larger the fibre, the faster the movement. Thus the chronaxie, which is the reciprocal of the rate, is 0·0003 second in the case of the motor nerve to the gastrocnemius, whose fibres have a diameter of 0·02 mm. and 0·02 second in the motor fibres to the stomach, with a diameter of 0·002 mm. It might, perhaps, be expected that the rate of movement would be greater in a large fibre, if the ions in the middle have to reach the membrane on the outside of the axis cylinder in the same time as those in a small fibre. But this is purely hypothetical. W. W. Waller (1914) finds that the optimal time value (Lapicque's "chronaxie") for excitation of sudo-motor nerves is ten times that for motor nerves to muscle.

Mode of Connection between Nerve and Muscle.—The balance of evidence is decidedly in favour of the view that there is merely close contact at the end-plate of the nerve; there is, apparently, no continuity of cell substance, but a membrane is interposed. When a nerve fibre degenerates, the process does not proceed beyond the end-plate. There is, no doubt, in excitation, a change at this "synaptic" membrane, by which the transfer takes place. The receptive substance of Langley must be supposed to lie on the muscle side of the membrane and be confined to the region in the neighbourhood of the connection with the nerve fibre. It is not necessary to regard this substance as a constituent radicle of the protoplasmic "molecule" of the muscle cell.

As we shall see later, a similar problem arises as to the transmission of excitation from the branches of one nerve cell, or neurone, to the cell body of another. We shall find that Sherrington's conception of a "synaptic membrane" is most in accordance with experimental facts.

INHIBITION

The name is applied to any process by which an action in progress is stopped by the application of some influence from without. It is not used when a process, such as a nerve impulse or a muscular contraction, excited by a momentary stimulus, runs a definite time course and then ceases spontaneously.

The result itself can evidently be brought about in different ways, according to the particular mechanisms involved; so that it does not seem correct to speak of a general theory of inhibition. Consider a smooth muscle cell in a state of natural tonus; excitation of a certain nerve fibre, connected to this cell, puts

an end to the contractile process and the muscle cell relaxes. A nerve cell may be in a state of excitation due to changes within itself, perhaps brought about by chemical influence outside, but the effect upon it of stimulation of a certain nerve fibre may be to quell the state of excitation or to make it inaccessible to the action of the chemical stimulant. Suppose, however, that the state of excitation is brought about by the reception of continuous stimuli brought in by nerve fibres making synaptic junctions with the cell in question. It is clear that the excitatory state of the cell could be abolished by making the synaptic membrane, of which we spoke above, impermeable to the excitatory process arriving by the nerve fibres; in other words, a block might be produced. In the case of the nerve cell, therefore, at least two different modes of inhibition are possible: the influences exciting it may be cut off, or the cell itself made incapable of responding to them, although they may be duly received. As another instance, we might take the ventricular muscle of the heart. It is conceivable that its natural beats might be stopped in several ways. The muscle itself might be made temporarily inexcitable, the rhythmic stimuli coming from the auricle might be prevented from reaching the ventricle by the production of a block on their course, or the contractile mechanism alone might be put out of gear, leaving the excitatory process intact, and so on.

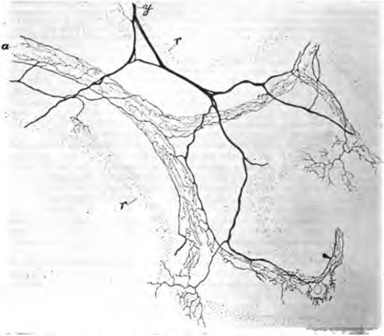


FIG. 109. PLEXUS OF NERVES AROUND A SMALL ARTERY OF THE SPLEEN. Golgi's method

a, Artery, surrounded by branched nerve fibres, which end freely.
y, Nerve from which the plexus arises.
r, Pulp substance. The broad white space around the artery is the Malpighian substance. In places nerve fibres are seen passing to the pulp.

(Retzius, 1892, Taf. XXI., Fig. 1.)

It is unnecessary to state that an actual destructive process is not spoken of as an inhibition. The possibility of return to the normal state, capable of again entering into a condition of excitation, is essential. In some cases, however, it may happen that a vicious circle is established, so that the normal state does not return, as we shall see later.

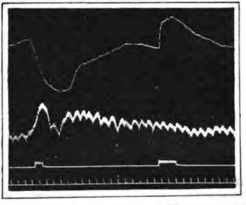


FIG. 110. EFFECT ON THE VESSELS OF THE TONGUE OF THE DOG OF STIMULATION OF THE VASO-CONSTRICTOR AND OF THE VASO-DILATOR NERVES.

Upper curve—volume of the tongue, obtained by a plethysmograph, so that a fall means decrease of volume, due to constriction of arterioles allowing a smaller amount of blood to be present; and vice versa when the curve rises.

Lower curve—general arterial pressure, showing that the changes in the tongue are not due to changes in the pressure at which the blood is supplied to it.

Upper of the two lines—signal, marking at the first rise, stimulation of the peripheral end of the cervical sympathetic nerve, which supplies vaso-constrictor fibres; at its second rise, that of the peripheral end of the lingual nerve, which contains the vaso-dilator fibres.

Bottom line—time in ten-second intervals.

As a general statement it may be taken that the processes at the basis of inhibition are of an opposite kind to those at the basis of excitation, so that the explanation of the one is involved in that of the other. If there is an increase of permeability in excitation, a decrease is to be expected in inhibition, for example. It will be found, however, that the definite knowledge we possess of the nature of the inhibitory process is still more defective than that of the excitatory process, so that all facts bearing on the question are of value.

To obtain a general view of the kind of facts to be explained, some experimental cases will be useful. They will also serve to indicate the different kinds of inhibition to which reference was made above.

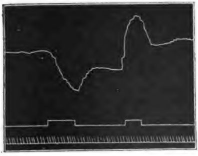


FIG. 111. TRACING FROM RETRACTOR PENIS MUSCLE OF THE DOG.

The fall in the curve is produced by lengthening of the muscle, brought about by stimulation of the pelvic nerve, which inhibits the spontaneous tonic state.

The rise is contraction, due to stimulation of the pudic nerve, which increases the degree of the tonic contraction.

(Bayliss and Starling.)

Smooth Muscle.—We have already seen how the intestine shows a series of rhythmic contractions, superposed on a background of moderate tonic contraction, and how these contractions and tone can be reduced by the splanchnic nerves and increased by the vagus nerves (see Figs. 96 and 97, page 369). Since the excitation of the spontaneous contractions proceeds from the muscle itself, the action of the inhibiting and exciting nerves is exercised on the muscle cells directly.

In other cases, as the muscular coat of the arterioles, there is no evidence that the plexus of nerve fibres supplying the contractile cells contains nerve cells (see Fig. 109), so that the state of tonic contraction is clearly inherent in the muscle itself. It can be increased by nerves called vaso-

constrictor, or diminished by those called vaso-dilator. Fig. 110 shows the fact in the case of the tongue. Stimulation of the sympathetic nerve causes contraction of the small arteries and therefore decrease of the volume of blood in the organ; that of the lingual nerve, which contains the vaso-dilator fibres, causes increase of volume. We have already met with another instance in the salivary glands.

The muscle called "*retractor penis*," present in certain animals, is a strip of smooth muscle arising from the anterior caudal vertebræ and inserted into the glans penis. It forms a very convenient object for the investigation of the properties of smooth muscle. Fig. 111 is a tracing obtained in some work (hitherto unpublished) in which Starling and myself were engaged. It is seen that the initial state of the muscle is one of moderate tonic contraction, since it can be relaxed by the stimulation of the pelvic autonomic nerves, and it can also be increased by stimulation of fibres contained in the pudic nerves.

Another instructive case is that of the large *pincers of the Crayfish*. There is here a powerful muscle closing the forceps, and a similar one opening it (see the figure in Huxley's book, 1880, p. 93). It is clear that, if the strong closing muscle remained in a state of contraction, the weaker opening muscle would be powerless. Accordingly, it is found that, if the nerve trunk supplying the appendage be excited in such a way that the forceps is caused to open, the closing muscle is inhibited, along with the contraction of the opening muscle, and, when the forceps closes, an opposite effect on the two muscles takes place. Richet (1882) showed that a weak direct stimulation of the claw caused opening, and a strong one caused closing, while Biedermann (1887, 1) showed that a weak stimulation of the nerve caused a contraction of the opening muscle, coincidently with inhibition of tone in the closing muscle, and that a strong stimulation had the opposite effect on both. The explanation of these facts seems to lie either in the existence of four sets of fibres in the nerve trunk, exciting for the closing muscle, inhibiting for the opening muscle, exciting for the latter, and inhibiting for the former; or two sets of fibres, each of which divides into two branches, one branch inhibiting the one muscle, while the other branch excites the antagonist muscle. The fibres causing contraction of the closing muscle and inhibition of the opening

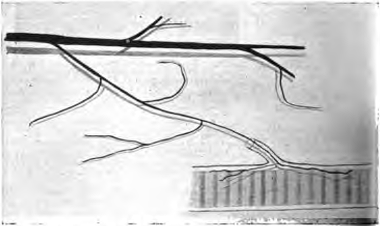


FIG. 112. INNERVATION OF THE CLAW MUSCLES OF THE CRAYFISH, ACCORDING TO THE WORK OF RICHET, BIEDERMANN, MANGOLD, ETC.—The two kinds of nerve fibres divide simultaneously and the terminal fibrils are distributed, one or more of each kind, to the same muscle fibre, passing inside the sarcolemma to end in the muscle substance.

one require a stronger current from the induction coil than the other set do, possibly due to their excitable substances having a different optimal rate of stimulation. It is a very suggestive fact that, as was shown by Biedermann at a later date (1887, 2), the fine branching nerve fibres in the muscles are always double, a slender fibre accompanying a thicker one, even to their endings in the muscle

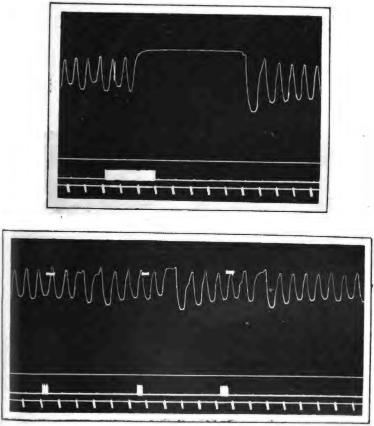


FIG. 113. ACTION OF THE VAGUS NERVE ON THE BEATS OF THE VENTRICLE OF THE TORTOISE.

Upper figure—On stimulation, marked by the signal, the beats cease, after a latent period, with a decrease of diastolic tone (the tracing lever pulls downwards). The effect lasts considerably longer than the stimulation, and is followed by an increase in the magnitude of the beats. The latent period is better shown in the lower figure, where the times of stimulation are marked by white rectangles on the heart tracing itself.

fibres. The same muscle fibre is supplied by a fine and a coarse fibre, and it is difficult to avoid the conclusion that one of these is excitatory, the other inhibitory, for the muscle cell. Fig. 112 is a diagram of the facts as made out by Biedermann and by Mangold (1905), who also investigated the question and found this plan of innervation to be a very general one in the Arthropods. It will be seen presently that the fact has an important bearing on the theory of inhibition.

The arrangement by which the *bivalve molluscs* open and close their shells was investigated by Pavlov (1885), who found that the powerful adductor muscle is

supplied with two sets of nerves, exciting and inhibiting. The natural state of the muscle appears to be one of tonic contraction, which can be reduced by certain nerve fibres. It then remains in this state until a stimulation of the exciting nerve fibres sets it into contraction again. Further details with regard to these tonus phenomena will be found in Chapter XVIII. It is important to note that, as Pavlov points out (pp. 28 and 29), there are no nerve cells in the muscle itself, so that the two kinds of influence must be exercised on the muscle cells directly. In view of later theories of receptive substances, it may be added that Pavlov states that there must be special parts in the interior of the muscle fibres on which the contraction depends, and others on which the relaxation depends.

The Heart.—In Fig. 113 tracings are given of the stoppage of the heart of the tortoise by stimulation of the vagus nerve. It will be noted that, in addition to cessation of the spontaneous beats, the state of tone in diastole is reduced. In Fig. 114 a curve of the action of the opposite kind of nerves, the accelerator or augmentor nerves, from the sympathetic system, is shown. More detailed analysis of the nervous regulation of the heart will be found in Chapter XXIII.

It was remarked above, that it is to be supposed that the phenomena associated with inhibition will naturally be of an opposite kind to those associated with excitation. In this connection, the observations made by Gaskell (1887) are of much interest. We have seen that the wave of excitation in muscle is accompanied by electrical negativity of the spot actually in the state of excitation. Now Gaskell showed how a preparation can be made of the auricle of the tortoise in such a way that spontaneous beats cease for a time, owing to separation from the place where these beats arise in the sinus, while the supply of inhibitory fibres is left intact in a nerve (the coronary), which is separate from the other tissues, and can be left when they are cut. We have also seen that if one electrode is situated on an injured spot, we obtain only the electrical change taking place at the other electrode on a normal spot. When the auricle contracts, then, there is a movement of the galvanometer needle in a direction indicating negativity of the auricular muscle under an electrode on a normal spot, when the other electrode is on an injured spot. Now, suppose that we excite the vagus nerve while the auricle is in a state of rest after separation from the sinus. Gaskell saw that the muscle becomes *electro-positive*; the galvanometer needle moved in the opposite direction to that associated with contraction (Fig. 115). It might, perhaps, be supposed that the explanation of this change is merely that the auricle, although showing no rhythmic beats, was in a state of slight tonic contraction, which would, of course, be associated with a permanent negative deflection of the galvanometer. If the vagus caused a disappearance of this tonus, the

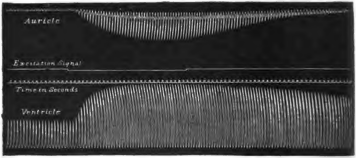


FIG. 114. ACTION OF THE SYMPATHETIC NERVE (AUGMENTOR) ON THE HEART OF THE TOAD.—Suspension method. Clamp in auriculo-ventricular groove. Sympathetic stimulated before it joins the vagus.

(Gaskell, 1900, Fig. 111.)

tissue would naturally become less negative, that is, it would appear to become electro-positive. It is of some importance, therefore, to note that Gaskell was unable to detect the slightest change in tonus, although using a very sensitive lever to magnify any possible movement. It is evident that inhibitory action is accompanied by some kind of change, which is of the opposite nature to that which is responsible for the negativity of a tissue in the state of excitation.

With regard to some doubts that have been expressed as to these results, I should like to state that I have myself on two separate occasions shown the fact as a lecture experiment and have obtained the electro-positive change without difficulty. It should be borne in mind that the magnitude of the change is much less than that of the negative change occurring on contraction, so that observers who have attempted to observe it with the capillary electrometer have been unable to do so. Recently, however, both Meek and Eyster (1912) and Samoilov (1913) have photographed it by aid of the string galvanometer, made especially sensitive. The advantage of the use of this instrument is that the positive effect can be seen in the beating heart, both of the frog and of the tortoise (see Fig. 116).

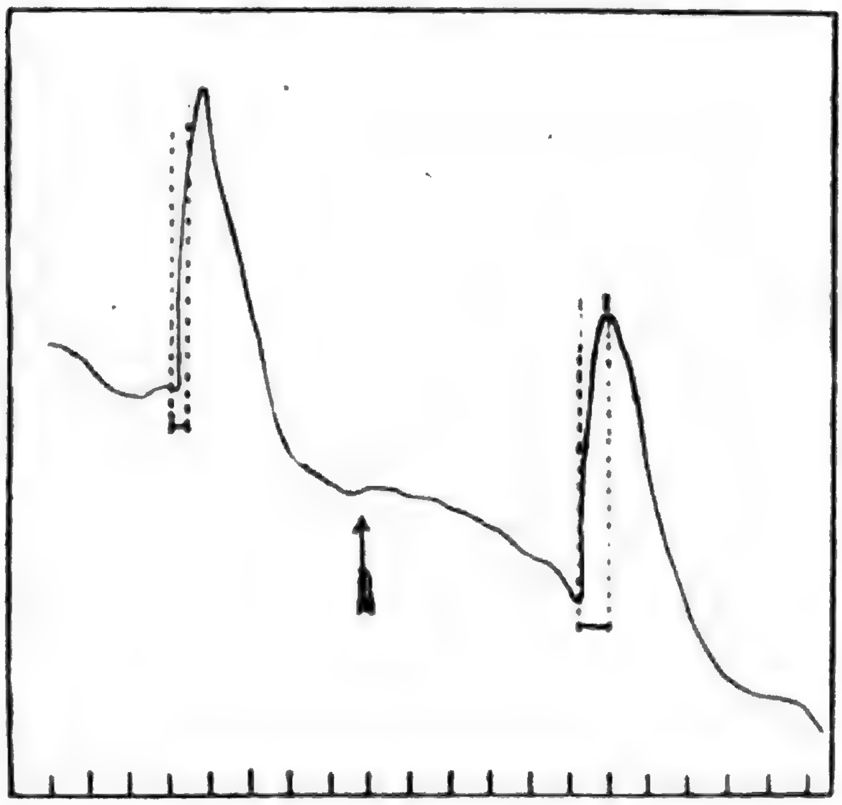


FIG. 115. ELECTRICAL CHANGE PRODUCED IN THE QUIESCENT AURICLE OF THE TORTOISE BY STIMULATION OF THE VAGUS NERVE BETWEEN THE DOTTED LINES ON THE CURVE.—At the arrow, muscarin was applied to the sinus to arrest its contractions. This has no effect on the subsequent stimulation of the vagus.

Note that the electrical change is opposite in sign to that associated with contraction, and also to that produced by the augmentor nerve in quiescent muscle (see Fig. 117 below).

(Gaskell, *Journ. of Physiol.*, **8** (1887), 404-415.)

Gaskell has also shown that, after the heart of the tortoise has been brought to a standstill by the application of the alkaloid muscarine to the sinus, stimulation of the vagus nerve produces the same change as that recorded above; moreover, stimulation of the augmentor nerves of the toad, which in the beating heart causes increase in the rate or height of the contractions of the ventricle, produces in the ventricle of the heart at rest under the action of muscarine or otherwise, an electrical change of the same sign as that associated with contraction of the muscle (Fig. 117).

Another effect of the state of inhibition is to depress the excitability of the heart muscle with regard to the response to direct stimulation. M'William showed (1885, 1) that the sinus, auricles and ventricle of the newt's heart are inexcitable under vagus inhibition, and (1885, 2, p. 226) that the auricle of the eel's heart behaves in the same way. Although this phenomenon could not be demonstrated in other animals, it shows that the action of inhibitory nerves is excited on the muscle itself.

The work of Dorothy Dale and G. R. Mines (1913) shows that the action of the vagus on the heart, as investigated by the string galvanometer, is to produce increased resistance to transmission from auricle to ventricle and to shorten the duration of the electrical disturbance. That of the accelerator nerves is to improve the rate of conduction and to increase the duration of the electrical response in the ventricle.

Since the effects of the excitatory and inhibitory nerves on smooth muscle are of an opposite nature, it is to be expected that the effect of one could be *balanced* by the simultaneous stimulation of the other. Experiments of this kind were

made by Reid Hunt (1897), who found that the result on the heart beat of simultaneous stimulation of the vagus and accelerator nerves was nil or minimal, if the effects when separately stimulated were equal and opposite. If the stimulation of the one was interpolated in the middle of that of the other, the rate of the beat was brought back towards that of the normal. In other words, the nerves are purely antagonistic and "the statement that a minimal stimulation of the one can completely overcome a maximal stimulation of the other is undoubtedly incorrect."

On the other hand, the experiments of von Frey (1876) are sometimes quoted in support of the opposite view. These experiments showed that, on *maximal* stimulation of chorda tympani and sympathetic nerves at the same time, the rate of blood flow through the submaxillary gland was slowed, showing that the constrictor effect overpowered the dilator one. When the stimulation ceased, the dilator effect of the chorda tympani showed itself to last longer than the constrictor effect of the sympathetic. By appropriate choice of the relative strength of the stimulation of the two nerves, Asher (1909) showed, however, that the effect of either dilator or constrictor nerves can be made to preponderate. Anrep and Cybulski

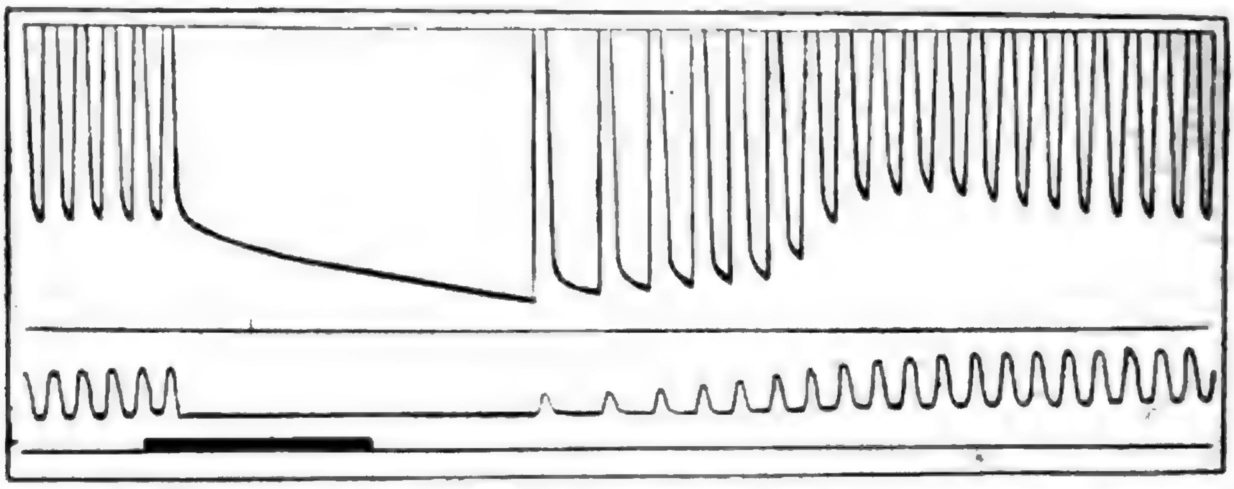


FIG. 116. POSITIVE VARIATION OF THE DEMARCATION CURRENT OF THE FROG'S VENTRICLE IN CONSEQUENCE OF STIMULATION OF THE VAGUS.

One electrode leads off from an injured spot.
String galvanometer.

Upper tracing—the monophasic electrical changes. The tops are too high to be included on the plate.
Below it—reference line.
Lower tracing—spontaneous heart beats, recorded by a lever.
On stimulation of the vagus, marked by the signal, there is a gradual fall in the diastolic level of the galvanometer line; that is, there is an electrical effect in the opposite direction to that associated with contraction. After this effect has passed off, there is a temporary rise in the level of the diastolic position of the string. Note that the diastolic position of the lever marking the beats shows no alteration during the course of the vagus inhibition.

(After Samoilov.)

(1884) found it easy to make either effect show itself in the case of the tongue. It is to be noted that von Frey's experiments were made to test a particular hypothesis, that of interference in ganglion cells, and the results are not in disagreement with the view of the supply of the same individual muscle fibre by two separate nerve fibres of opposite action.

Secretion.—If the view taken in our chapter on Secretion be correct, namely, that, up to a certain stage, this process is an automatic one on the part of the cell mechanisms, there is some reason to suppose that there might be nerves inhibiting the process. In the case of the pancreas we have met with some evidence, brought forward by Pavlov and his co-workers, that the vagus nerve contains inhibitory fibres for this gland. One way in which this influence can be seen is the following: suppose that we have, by repeated periods of stimulation of the vagus, obtained a flow of juice; this flow outlasts the actual period of stimulation, and, if the stimulation be applied to the vagus afresh before the effect of the preceding stimulation has ceased, it will be seen that the first effect is a cessation of the flow, which is afterwards followed by an increase. The reader may remember that this is very similar to the phenomenon seen in the action of the same nerve on the

movements of the intestine, so that it is not unnatural to imagine that the effect on the pancreas might be due to the relaxation and contraction of muscular fibres in the ducts. That this is the explanation is shown by the experiments of von Anrep, described on page 349 above.

Jappelli (1908) states that, if an attempt is made to produce reflex salivary secretion by exciting the central end of the lingual nerve, no effect is produced as long as the stimulation lasts, but that the secretion appears subsequently. If secretion is in progress when the nerve is excited, a temporary cessation occurs. Before we can accept these results as evidence of inhibitory fibres, experiments on the blood flow through the gland must be made under the conditions of the experiment. It is practically certain that reflex vaso-constriction was produced in the gland by stimulation of the sensory nerve and the failure of blood supply would be quite sufficient to account for the results.

Reference was made previously (page 349) to the "anabolic" nerves supposed to supply the gland cells; the function of these fibres is supposed to be to excite the building-up process in these cells. According to the theory proposed by Gaskell, to be discussed presently, anabolic nerves are inhibitory. When the chorda tympani is cut, these tonic inhibitory impulses are removed and we have "paralytic secretion." The basis of this theory will be found not to be a very solid one.

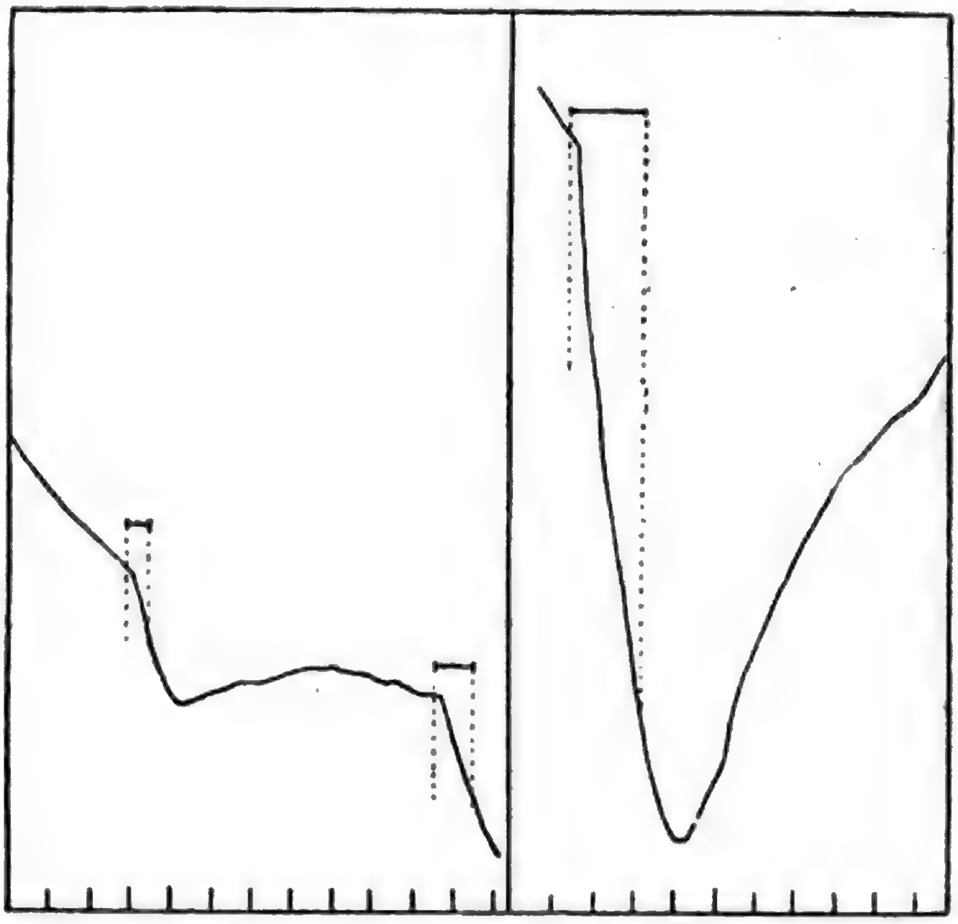


FIG. 117. ELECTRICAL CHANGE PRODUCED IN THE QUIESCENT VENTRICLE OF THE TOAD BY STIMULATION OF THE AUGMENTOR NERVE.—The first two stimulations, after the heart had been brought to a standstill by application of muscarin to the sinus. The last one, on another heart after the ventricle had ceased to beat owing to block of impulses from the sinus by application of a clamp between the auricle and ventricle.

The sign of the electrical effect is similar to that of spontaneous contraction, and opposite to that produced by the vagus.

(Gaskell, *Journ. of Physiol.*, **8**, 404-415).

Nerve Centres.—We come now to a very important region in which inhibitory processes play as fundamental a part as those of excitation. A few examples will be given here, and the nature of the phenomena discussed later.

The necessity of the process will be apparent, if we consider the state of affairs in a complex co-ordinated act, in which the nerve cells of centres governing various muscles are called upon. Some or all of these cells are certain to be occupied in other ways at the time; that is, they are already in a state of excitation due to the arrival of messages from other sources. If they are to perform the new process properly, they must be freed from this previous state of excitation, so as to be accessible to the fresh one. Further, the activity of certain centres sets into contraction muscles which oppose those required in the new act and which must be relaxed.

In Fig. 107, on page 388 (from Sherrington and Sowton, 1911, p. 443), we saw that a state of tonic reflex contraction is produced in the vasto-crureus muscle, by stimulation of the central end of the popliteal nerve with rheonome currents. This tonic excitation of the nerve cells continues after the stimulus has ceased, as shown by the line of the signal at the base of the figure. At the second mark of the signal, a weak tetanising stimulation was applied to the same nerve. The tonic state of the nerve cell is completely abolished, but returns when the inhibiting stimulus ceases

Fig. 118 (from Sherrington, 1905, p. 277) shows some further points. In this case, the reflex contraction of the muscle was brought about by stimulation of the skin of the foot of the opposite side, indicated by the lower signal. At the rise of the upper signal, the foot of the same side as that of the muscle observed was stimulated. The excitation of the centre disappears, and it is to be noted that it falls below the level at which it was before the reflex contraction was induced. The cells of the centre were, therefore, already in a state of partial tonic excitation before the exciting stimulus was applied, and this was abolished along with that due to the stimulation of the afferent nerve.

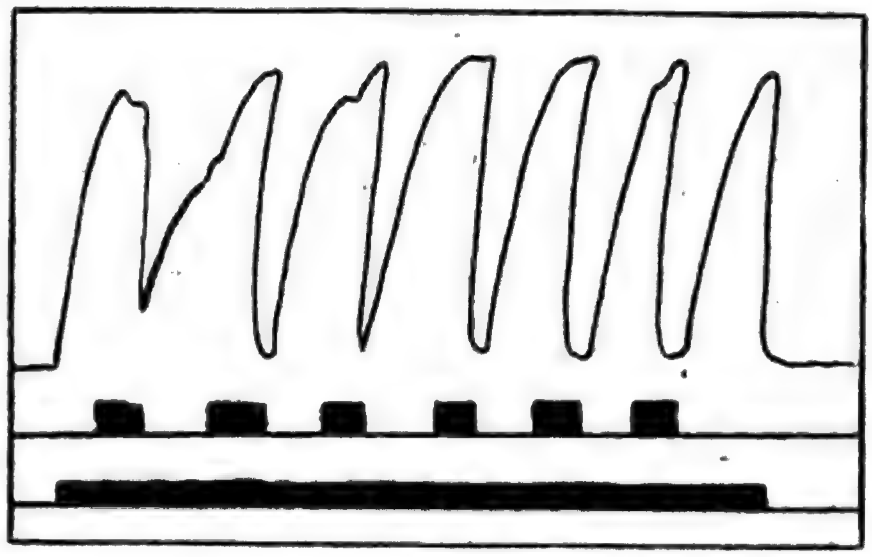
FIG. 118. REFLEX INHIBITION OF DECEREBRATE TONE.—The tone was induced by stimulation of the skin of the foot of the opposite side, marked by the lower signal and by lines cutting the myogram. During the time marked by the upper signal, the foot of the same side as the muscle was stimulated. Note that the muscle was, to begin with, in some degree of tonic contraction, and that this is inhibited together with that due to the reflex from the contralateral foot. The muscle falls to the position of complete relaxation.

(Sherrington, 1905, Fig. 3.)

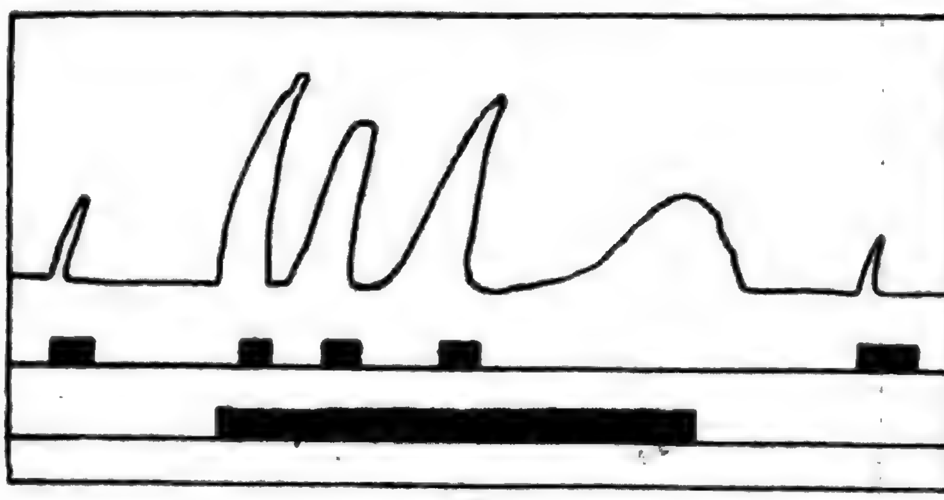
Fig. 119 (Vészi, 1910) is a somewhat simpler case, being a reflex from the spinal cord separated from the higher centres. The gastrocnemius muscle of the frog is put into continuous reflex tetanus, by stimulation of the afferent dorsal root of the 9th spinal nerve, during the mark of the lower signal. At the several marks of the upper signal, the central end of the 8th root was stimulated. There is complete

inhibition of the centre, although the stimulation causing reflex contraction was continued.

We may take another example from a different group, the vasomotor reflexes. In Fig. 120 (from an experiment of my own), the uppermost curves represent the volume of one of the hind legs of a dog, a rise in the curve meaning increase of volume due to the presence of more blood in the limb. The vaso-dilator supply had been cut off from the centre by section of the spinal cord in the upper lumbar region. Between the two parts of the tracing, the vaso-constrictor supply was also cut off by section of the abdominal sympathetic chain. The effect of this, as will be seen, was to cause an increase in the volume of the limb, although there was no change in the blood pressure (the first part of the tracing was interrupted before the blood pressure had returned to its normal level, which, at the time of section of the sympathetic, was at its initial height as at the beginning of the figure). The only way in which this vaso-dilatation could have happened was that the vaso-constrictor centre was in a state of tonic excitation, and sending impulses to the limb, keeping its arterioles in a state of tonic contraction. Section of the efferent nerves conveying these impulses released the blood vessels from this tonic excitation, and pressure of the blood inside them caused expansion. At the two marks on the signal line, the central end of the vagus nerve was stimulated. In the dog there are, in this nerve, fibres which excite and also fibres which inhibit the vaso-constrictor centre, but the fall of blood pressure, in the lower curve, shows that in this case the effect of the latter, the "depressor" fibres, was present alone. Owing to inhibition of the tonic state of excitation of the vaso-constrictor centre, the limb dilates in the first stimulation, since the arterioles are freed from the constrictor impulses, just as by the subsequent section of the nerve fibres themselves. The second stimulation, after section of the sympathetic



A



B

FIG. 119. INHIBITION OF SPINAL REFLEX IN THE FROG.—All sensory roots of the sciatic nerve cut.

A, Upper tracing—contractions of the gastrocnemius muscle.
Lower signal—continuous stimulation of the central end of the 9th dorsal root.
Upper signal—intermittent stimulation of the central end of the 8th dorsal root.
The reflex contraction due to the stimulation of the one root is inhibited every time that the other root is stimulated.

B, Similar experiment in which the action of the 10th root is inhibited by stimulation of the 8th root.
The first and last contractions on the myogram show that stimulation of the 8th root by itself alone causes reflex contraction, although brief.

(After Vészi.)

chain, is merely to show the absence of any direct effect on the vessels of the limb, the fall in the curve being due to the diminution in the blood content, owing to the lowered blood pressure produced by dilatation in the blood vessels of those other parts of the body still under the dominion of the vaso-constrictor centre.

Experiments on *balancing* excitation against inhibition in nerve centres show that, by properly choosing the relative strengths of the stimulation applied to the respective nerves, an exact neutralisation can be obtained. I found (1893, p. 318) this to be the case with the vaso-constrictor centre and give an example in Fig. 121. The tracing is that of the arterial pressure in the rabbit. During the rise of the upper signal, the depressor nerve was stimulated continuously,

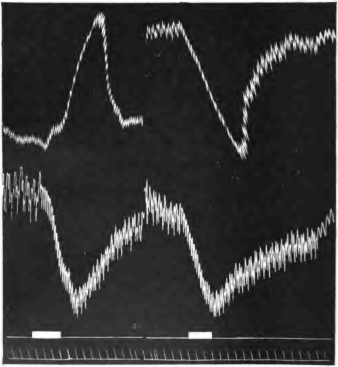


Fig. 120. Depressor reflex on the arterioles of the hind leg from stimulation of the central end of the vagus in the dog.—Vaso-dilators cut.

Upper curves—volume of the leg. Rise indicates vaso-dilatation.
Lower curves—arterial pressure. Zero is 24 mm. below the time signal.
Time in ten seconds.

The first stimulation shows vaso-dilatation produced by inhibition of tone in the vaso-constrictor centre. The arterial pressure falls. Before the second stimulation the abdominal sympathetic nerve was cut. The fact that this nerve was conveying tonic vaso-constrictor impulses is shown by the rise of the level of the volume of the leg seen in the tracing.

The second stimulation produces a similar fall in arterial pressure, but the leg, instead of expanding, merely follows the blood pressure; decrease in volume is due to the lower pressure at which the blood is driven in and to its being drained away by vascular dilatation in other parts. No inhibition of constrictor tone could be shown by the leg, since the vaso-constrictor nerves, passing to it from the centre by way of the sympathetic, were divided.

(Bayliss, 1908, 2, Fig. 4.)

producing its normal fall of blood pressure; this fall is interrupted, temporarily, during the rise of the lowest signal line, by a return to normal, resulting from simultaneous stimulation of an ordinary afferent nerve, the anterior crural, which, on its own account, would have produced a rise of arterial pressure above normal by excitation of the constrictor centre. The effect of this nerve lasts longer than the period of stimulation, but the continued stimulation of the inhibiting

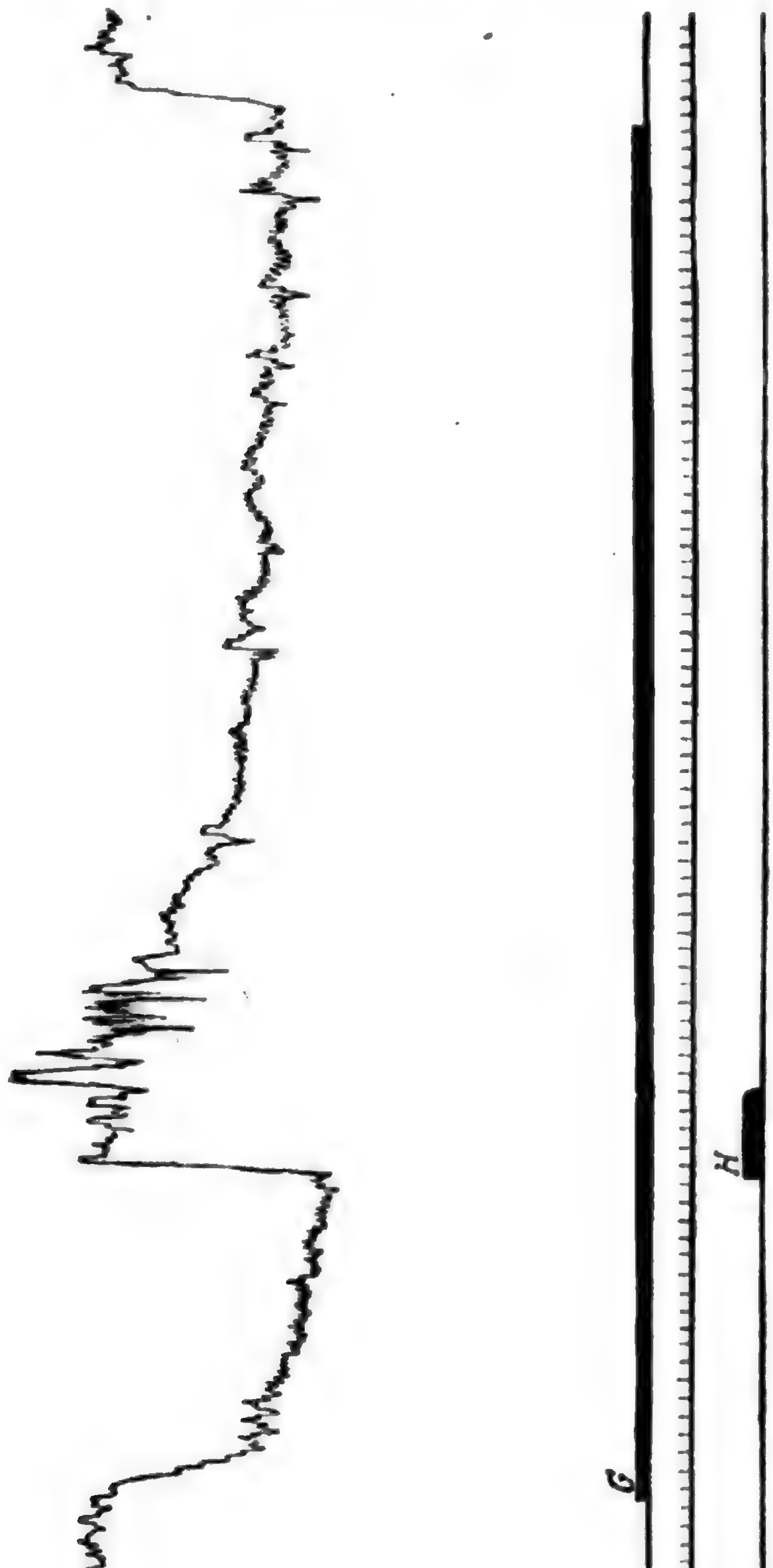


FIG. 121. BALANCE OF EXCITATION AND INHIBITION IN VASO-MOTOR REFLEX.—Rabbit, arterial pressure curve.

Signal *G* marks stimulation of the central end of the depressor nerve.

Signal *H* marks stimulation of the central end of the anterior crural nerve (pressor).

Time in twelve-second intervals.

The normal level of the arterial pressure is that at the beginning of the curve. At *G*, depressor stimulation causes it to fall. At *H*, concurrent stimulation of the pressor nerve brings it back to normal. After the effect of this brief period of stimulation has passed off, the depressor effect returns and the pressure does not become normal again until the stimulation of this nerve ceases. The relative strength of the stimulations of the two nerves was, by chance, that just needed to balance each other exactly. It is unnecessary to add that the anterior crural nerve alone would have produced a rise above normal.

(Bayliss, 1893, Fig. 21.)

nerve makes itself felt again and the blood pressure rises finally to normal only when the stimulation of the depressor ceases.

In Fig. 122 two tracings are given from Sherrington's paper (1908) which show the same fact in the case of reflexes to skeletal muscle. The vasto-crureus muscle of a decerebrate cat traces its contraction by a rise in the curve. The upper signal indicates by a fall the stimulation of an inhibitory nerve, the central end of the peroneal of the same side, and the lower signal that of an exciting nerve, the central end of the popliteal of the opposite leg. In A, the inhibiting

nerve is first stimulated and the muscle is released from the tonic excitation of the centre. In the middle of this period of stimulation, the exciting nerve is stimulated, the inhibition of the centre is almost neutralised, so that the muscle returns nearly to its original length. While this stimulation is continued, the inhibiting stimulus is removed, with the result that the exciting stimulus can produce its full effect unhindered. In B, the converse experiment is performed, commencing with the stimulation of the exciting nerve. Sherrington concludes that the effect is a simple algebraic summation of the two single effects (see also

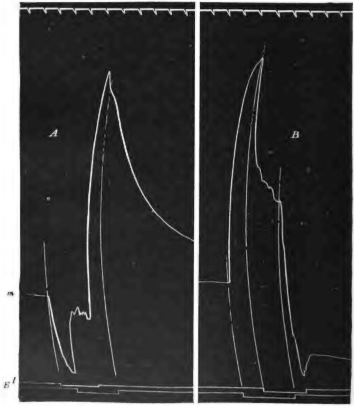


FIG. 122. BALANCE OF REFLEX EXCITATION AND INHIBITION IN THE CASE OF SKELETAL MUSCLE (VASTO-CRUREUS).

In the left-hand tracing (*A*) the myogram (*m*) shows that medium (decerebrate) tonus is present at the commencement. The fall of the signal line *I* indicates that the central end of an inhibitory afferent nerve, the ipselateral peroneal, is excited by 150 units of the coil graduation. The level falls. It is brought back again nearly to its original height by concurrent stimulation (shown by signal *E*) of an excitatory nerve, contralateral popliteal, with 500 units. When the inhibitory stimulus is cut off, the full effect of the excitatory one shows itself.

In the right-hand tracing (*B*) the order in which the two nerves are stimulated is reversed.

Note the greater rapidity of the final fall in *B* than in *A*. This is due to the fact that in *B* it is produced by active inhibition, in *A* by mere cessation of excitation.

(Sherrington, 1908, Fig. 4.)

Fig. 123). In the case of the vasomotor reflexes I stated the same conclusion thus: the results "depend entirely on the relative strengths of the stimuli; whichever nerve is under the stronger excitation shows its own effect, and when the excitation of this one is stopped, the effect of the other manifests itself. In fact, there appears to be a perfect antagonism" (1893, p. 318).

"*Inhibition of Inhibition.*"—Suppose that we have in tonic activity the centre from which nerves proceed which cause inhibition or relaxation of the smooth muscle which they supply. Such a case is that of the vaso-dilator centre under certain conditions, as will be seen later. Now there are afferent nerves which inhibit this centre, just as the depressor nerve inhibits the vaso-constrictor centre, so that we have, as Sherrington has called it, an "inhibition of inhibition." In Fig. 124 we have, in fact, a case which is incapable of explanation on any other view. This is from an experiment in which I was engaged in investigating the blood flow through the submaxillary gland of the rabbit. It will be seen that, when the central end of the vagus nerve of the opposite side was stimulated, there was a longer interval between the drops of blood from the vein, that is, the arterioles were narrowed. Now the vagus nerve in the rabbit is a pressor nerve, that is, it causes a rise of arterial pressure by reflex constriction of arterioles. This rise of pressure is not shown on the tracing, because an arrangement was used which kept the general blood pressure at a constant level by allowing blood to

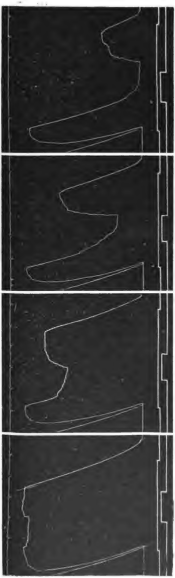


FIG. 123. ALGEBRAIC SUMMATION OF CENTRAL EXCITATION AND INHIBITION ACCORDING TO RELATIVE STRENGTH.—Reflex contractions of the semitendinosus muscle, evoked by stimulation of the ipselateral peroneal nerve. (Note that this nerve produces inhibition of the antagonist muscle, vasto-crureus, in the previous figure.) The intensity of the stimulus to this nerve is the same in all the four observations. In each, the contraction is inhibited to a different degree by intercurrent stimulation of the contralateral popliteal nerve (lower signal), the intensity of the stimulus being increased progressively from left to right.

Decerebrate cat. Time in seconds above.

(Sherrington, 1909, 2, p. 254.)

escape (see my paper, 1908, 3, p. 272). Hence, if this cervical sympathetic nerve, conveying constrictor impulses to the gland vessels, had been intact, explanation would be simple, namely, by reflex excitation of vaso-constrictor nerves. But, in point of fact, the vaso-dilator fibres of the chorda tympani were the only vascular nerves left intact, so that the effect must have been produced through these and indeed by inhibiting a previously-existing tonic state of an inhibitory centre. It may be noted that one inhibition acts on muscle, the other on nerve cells.

It is shown by the work of Pavlov on "Conditioned Reflexes" (see p. 506) that similar phenomena play an important part in the mechanism of the nerve centres themselves. Suppose that a motor nerve centre is inhibited by the action upon it of an inhibiting nerve, acting through an intermediate neurone. In other words, the state into which this intermediate neurone is put by the stimulation of a particular afferent nerve is such as to give rise to inhibition of the motor neurone. Now it is quite possible that another nerve, acting on this intermediate neurone, might inhibit it; in which case, the motor neurone previously kept in check by the intermediate neurone would be set free, and apparently excited by a nerve which was really inhibiting another neurone. The state of excitation of our motor neurone is, of course, assumed to arise from some independent cause, inherent, reflex, or chemical. We shall see presently that an inhibitory nerve can antagonise a chemical excitation as well as a nervous one, and, perhaps, the distinction ought not to be made in this form, since the ultimate process is probably identical in both cases, as remarked above (page 344) (see Bayliss, 1919, 3, diagram on p. 121).

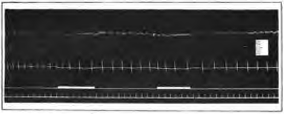


FIG. 124. CENTRAL INHIBITION OF EXCITATION OF INHIBITORY NERVES, THAT IS, OF NERVES WHICH RELAX TONUS IN PERIPHERAL MUSCLES. INHIBITION OF THE VASO-DILATOR CENTRE.—Rabbit.

Upper tracing—arterial pressure. Zero at level of upper signal. Scale in millimetres.

Top signal—drops of blood from vein of submaxillary gland.

Middle signal—two stimulations of the central end of the vagus of the opposite side (pressor nerve).

Bottom signal—time in ten seconds.

The vagi, sympathetic, and depressor nerves were cut on both sides, so that the gland vessels were supplied only by dilator nerves in the chorda tympani nerve.

Stimulation of the vagus produced no rise of blood pressure, because the animal was eviscerated and the aorta was connected with an automatic arrangement, by which rise of pressure was prevented. We note that although there was no vaso-constrictor supply, yet vascular constriction was produced in the submaxillary gland, as shown by the longer intervals between the drops of blood escaping. Since the only vasomotor nerves were dilators, constriction could only be caused by inhibiting the impulses from the centre which kept these nerves in a state of tonic stimulation.

"Shock" and "Decerebrate Rigidity."—When the spinal cord is cut across, it is found that the portion posterior to the section remains for some time in a state of diminished excitability, so that no reflexes can be obtained from it. This state of "shock" is gradually recovered from. It has been supposed to be due to some effect of the injury, but Trendelenburg (1910) has made some experiments which indicate that this is not the explanation. Instead of cutting through the cord, he abolished its power of conduction by local cooling to a point just above its freezing point. The experiments were done on the rabbit, since the blood supply of the cord in this animal is such as to preclude the possibility of cooled blood passing to the posterior part of the cord and affecting its excitability directly. It was found that functional separation of the lumbar cord from the higher parts of the central nervous system could be made without any excitation. Notwithstanding this, the reflex excitability of the lumbar cord was abolished, but returned again when the temperature of the cooled segments

was allowed to return to the normal height. It seems that the "shock" effect must be due to the removal of some influence exerted by the higher parts of the nervous system, an influence of such a kind as to increase the excitability of the cord. Sherrington (1906, pp. 240-248) had previously come to the conclusion that spinal shock has nothing to do with the injury of the section, and that it was probably caused by some influence exerted by the mid-brain on the lower centres. He shows that, when recovery from a transection of the cord has taken place, a second transection just below the first has practically no effect.

The work of Pike (1909, 1912, 1913) shows that the phenomena are not due to the traumatic stimulation of long inhibitory tracts from the higher centres to the spinal cord. He looks upon the normal path for what are called spinal reflexes as having been diverted in the course of evolution, so that the impulses do not pass directly across the cord, but ascend to the higher centres first and from these return to the motor neurones. The direct path has thus become more or less impassable from disuse, but it can, after a time, regain the power of conduction to a certain extent, when the path through the higher centres is cut. This point of view will be better understood when Chapter XV. on the nerve centres has been read.

Decerebrate Rigidity.—Sherrington found (1898, 1906, pp. 299-303) that when the crura cerebri are cut through, the centres of certain groups of muscles are so much increased in excitability that the ordinary slight stimuli arising from the periphery are sufficient to maintain these muscles in a state of reflex tonic contraction. The centres in question are situated somewhere between the crura and the lower part of the spinal bulb, but not in the cerebellum. Under normal conditions, their excitability is restrained by the inhibitory influence of the cerebral cortex.

Weed (1914) comes to the following conclusions as the result of extensive investigation. The main reflex centre for decerebrate tonus is in the mid-brain, probably the red nucleus. The cerebellum, although not the absolutely essential pathway for the afferent impulses concerned, is the most important one. It is also the link in the inhibitory channel from the cerebral cortex, which prevents this rigidity in normal conditions.

This condition of "*decerebrate rigidity*" is very useful in the investigation of reflex inhibition, since we have centres in a state of tonic excitation, which can be played upon by the stimulation of various nerves. We have seen an example of this in Fig. 118 (page 410).

The Action of the Anode.—We have seen that excitation proceeds from the cathode. The simplest way of showing the fact is by the use of the device of J. S. New (1899).

Since excitation is associated with increased concentration of cations at a particular membrane, it is natural to suppose that the effect of the anode, being to decrease this concentration, would result in inhibition. It is clear, however, that this cannot show itself unless the tissue is in a state of excitation when the anode is applied. Biedermann (1895, p. 227) has made use of the drug, veratrine, to produce this state of excitation in voluntary muscle. A muscle under the influence of veratrine gives a prolonged contraction in response to a single stimulus and, during this state, a current can be sent through the muscle in such a way that the effects of the anode and cathode can be observed separately. It was found that the anode causes relaxation, the cathode, increased contraction. A still simpler way of seeing the fact, as Biedermann points out (1895, p. 219), is to place the anode on the beating ventricle of the frog's heart, the cathode on some other part of the body. It will be seen that the neighbourhood of the anode remains permanently in a state of relaxation, while the remainder of the ventricle undergoes periodic contraction.

Another aspect of the action of the anode is manifested in the case of nerve. The exciting effect of a constant current is only shown when it is made or broken; in other words, while it is really constant no excitation occurs. It must change its strength at or above a certain minimal rate in order to excite. But the nerve is not unaffected during the period of a constant flow of current. In the

neighbourhood of the cathode, a propagated disturbance can be set up by a smaller stimulus than in the normal state, and in the neighbourhood of the anode a stronger stimulus is required. The excitability is increased at the cathode; diminished at the anode.

Chemical Agents.—We see then that inhibition, as well as excitation, can be brought about by means other than stimulation of nerves.

Various chemical agents, as we saw in discussing the action of electrolytes in general, can produce a state of inexcitability. The hypothesis of Howell, as to the function of potassium in the action of the vagus nerve, was also referred to in that connection.

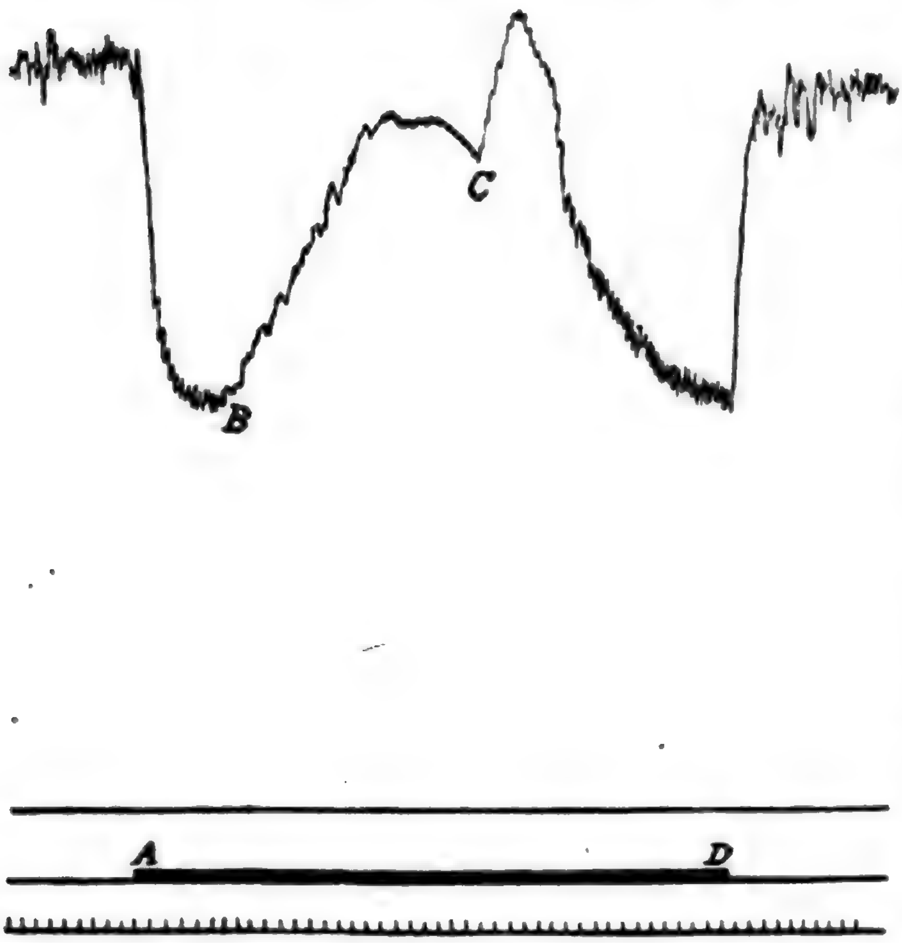


FIG. 125. ANTAGONISM OF DEPRESSOR INHIBITION AND ASPHYXIAL STIMULATION OF THE VASO-CONSTRICTOR CENTRE. —Curarised rabbit. Arterial pressure curve.

Depressor stimulated from *A* to *D*.

The pressure would have remained throughout at the level of *B* if the artificial respiration had not been stopped at this point. The result of the asphyxial excitation is to bring back the arterial pressure to normal. Respiration was resumed at *C*, and, as the asphyxial state disappears, the depressor fall again shows itself until the final cessation of the stimulus to the nerve.

(Bayliss, 1893, Fig. 24.)

It is interesting to note that excitation by chemical means can be antagonised by nervous inhibition, as shown in Fig. 125, which shows that the excitation of the vaso-constrictor centre by asphyxial blood is neutralised by simultaneous stimulation of the depressor nerve (see Bayliss, 1893, p. 319).

Fig. 126 is another interesting case. The tracing is that of the respiratory movements of the slip of the diaphragm in the rabbit which is attached to the end of the sternum, as used by Head for the investigation of respiratory reflexes. At the commencement of the figure it is completely relaxed and at rest, the vagus nerves being intact. The series of contractions was caused by the administration of air containing carbon dioxide. At × the vagus nerves were cut; the muscle is seen to pass into a state of partial tonic contraction, owing to excitation from the respiratory centre, which had previously been inhibited by impulses from the vagus endings in the lungs. A second inhalation of carbon dioxide excites respiratory movements of the slip, and the point of interest is that its tonus is inhibited, so that in expiration the position is that of nearly complete relaxation.

THEORIES OF INHIBITION

The facts brought out in the several cases described above will, as it seems to me, serve to show that it is not to be supposed that the inhibition of a process is in all cases effected in the same way. A general theory of inhibition is thus improbable, although there is no doubt that all the cases have the same essential basis and, when we consider that the process of inhibition is the opposite of that of excitation, we realise that when we know more about the latter, we shall know

more about the former also. It is to be feared that the consideration to be given to certain theories of inhibition will leave us with very little of definite value. It is necessary to do this, however, since some theories, proposed as of more or less general application, will be found to be inapplicable to any case whatever. It appears, on superficial examination, that we must consider the possibility of two different modes of inhibition. When a smooth muscle is in a state of tonus, due to its own inherent properties, the action of an inhibitory nerve is to quell this state of excitation by acting on the excitable substance of the muscle directly. When the state of excitation is due to the action of an excitatory nerve fibre upon the muscle cell, it would seem more appropriate to block the point of synapse where the nerve fibre conveys the excitation to the muscle. Similarly, a nerve cell may be in a state of excitation, due to chemical changes in itself or to chemical influence of constituents of the blood, and, in this case, inhibition may be brought about by action on the actual cell substance. If, on the contrary, the state of excitation is due to nerve impulses acting through synapses with the cell, it would seem to be a simpler hypothesis to suppose that the action of an inhibitory nerve fibre is exercised on the synapse, making it impermeable to the excitatory impulses. The cell substance itself is thus left free to respond to other stimuli. It does not seem impossible that experiment may decide whether there is any real distinction of this kind, and it is quite likely that it is the synapse that is concerned in all cases. Sherrington (1906, p. 103), in fact, is inclined to regard the process of inhibition as of the same fundamental nature in all cases, whether on nerve cell or on peripheral muscle.

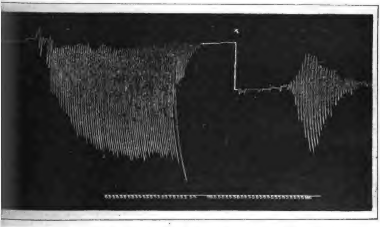


FIG. 126. **TONUS OF RESPIRATORY CENTRE AND EFFECT OF THE VAGUS NERVE THEREON.**—Tracing of diaphragm slip of rabbit by Head's method (see page 418).

Movement downwards indicates contraction of the diaphragm.

At the beginning, with the vagus nerves intact, the slip was completely relaxed, showing very small respiratory contractions. The series of large contractions was produced by the addition of carbon dioxide to the air breathed.

At ×, the vagi were cut. It will be noted that the diaphragm passed into partial tonus.

A second inhalation of air containing carbon dioxide caused increased respiratory movements, and it is interesting to note that, between each respiration, the tone was relaxed.

Time marker in seconds at the bottom.

(Obtained by Prof. Starling in a demonstration.)

Physical Interference.—Although it is possible that this view is not seriously advocated at the present time by any competent physiologist, there are still traces of it to be found, especially in certain work of the Verworn school. We

must, therefore, devote a few words to it. It is a familiar fact that a wave motion can be annulled by another similar wave motion of the same period, if the phase difference is of half a wave length. In this case, the hollows produced by the one are exactly filled up by the crests of the other. But it is essential to remember that these wave motions are, both of them, movements to alternate positions at an equal distance on *opposite* sides of a mean position, and that it is to this mean position that the process is reduced when the two wave motions mutually counteract one another.

For example, take an alternating electric current, as perhaps most analogous to a nerve impulse. This raises the potential of a point on a conductor alternately, say, to 100 volts above the potential of the earth and 100 volts below this value. Another similar current, with a phase difference of half a wave length, reduces the potential to a constant one of that of the earth, that is to zero.

Now, in a series of nerve impulses, the state of excitation is merely raised periodically from zero to one of a certain potential, and by no possibility could another similar set of disturbances reduce this potential to zero. In the most favourable case, it could be reduced only to one-half of its value. Inhibition, as Sherrington points out (1906, p. 99), is complete, and we see the fact in Figs. 107, 113, and 118. This could only happen, on the assumption of a process analogous to physical interference, if we could apply a nerve process of the opposite nature to that of excitation. This is just the fact which has to be explained! We may, then, dismiss the hypothesis as inadequate.

It will be clear that if a nerve is stimulated at the same moment at two points

A and B, at equal distances on opposite sides of the two leading off electrodes, C and D, the waves arriving at C and D simultaneously will produce an equal fall of electrical potential at the two electrodes, so that no external change will be obvious. It is incorrect, however, to speak of this as an "interference," as is sometimes done. It is also unnecessary to point out that the non-effect of a stimulus falling in the refractory period, or the disappearance of a disturbance in a region of decrement, cannot be spoken of as interference in any physical sense.

In a certain sense, the refractory period after an excitation is itself an inhibition, since the excitable tissue is incapable of entering into activity; so that there is some superficial resemblance to inhibition when a stimulus applied in this period is ineffective. But it does not quell a state of excitation already present, as a true inhibition does.

At the same time, the phenomenon known as the "*Wedensky inhibition*" requires a little consideration. Although this is a somewhat special case, it is of interest as showing the complex possibilities involved in joint action of refractory period and "all-or-nothing" law, as worked out by Adrian (1913). At a certain stage in narcosis or fatigue of a nerve-muscle preparation, a rapid series of strong stimuli applied to the nerve produces a small initial twitch only, whereas a similar series of weak stimuli produces a continued tetanus. After the first stimulus, in either case, a refractory period is present; if the next succeeding stimulus is strong, it will set up a propagated disturbance early in the relative refractory state, but this will only be a very small one and consequently unable to pass the region of decrement between the excited spot and the muscle. This region of decrement may be in the place of synapse between the nerve and the muscle, or in a narcotised area of the nerve itself. Although it is a small disturbance, it will leave behind it a refractory period, which will have the corresponding effect on the next succeeding stimulus and so on; thus no excitation will reach the muscle. If the stimuli are weak, on the contrary, no propagated disturbance can be set up by any one until the refractory period is practically at an end, and then the disturbance, although set up by a weak stimulus, will be of the full normal magnitude and able to pass the region of decrement and excite the muscle. Further details will be found in the original paper; those given above will suffice for our present purpose.

The experiments of von Frey (1876), previously referred to, were undertaken to test the hypothesis of an interference process, in which the excitation and inhibition were supposed to act on the same cell mechanism. It was found that, if the vaso-constrictor and vaso-dilator nerves to the submaxillary gland were stimulated simultaneously, the former obtained the victory during the period of stimulation, but the latter showed their effect afterwards. Now, although this result is inconsistent with a purely physical interference process, which should

result in a total abolition of the dilator effect, it does not disprove the hypothesis that the excitatory and inhibitory nerves do actually play on the same muscle cell or nerve cell, although not in the way of interference of wave motion. It will be found, in fact, that experimental results necessitate this view. We must remember that, at the time of von Frey's experiments, it was not known that the activity of the gland cells gives rise to certain products, "metabolites," possibly of an acid nature, which diffuse to the arterioles and, acting there as chemical agents, cause dilatation. This, naturally, continues its action after the actual stimulation of the nerve has ceased. Attention has been directed to it chiefly by the work of Barcroft (1907).

In cases where a process is a spontaneous one, it will be clear that it will resume its activity on cessation of the inhibitory influence.

There is more evidence in favour of a second group of theories, founded on the *nutrition* of cells. The foundation of this view was laid by Hering in his papers on sensations of light (1878) and worked out in more detail in a celebrated paper in "Lotos" (1889). It rests on the idea of the opposition between assimilation and dissimilation in Hering's words, or anabolism and catabolism in those of Gaskell (1886, p. 46). Verworn has adopted it and elaborated it in connection with his biogen theory.

There are, however, many objections to be brought against the theory. In the first place, we may consider the main principle. When a cell is actually increasing in the substance of its protoplasmic machinery, there is no doubt of the fact that it is engaged in building up complex systems out of simpler food materials; this is assimilation or anabolism; it may be considered as analogous to the manufacture of a petrol motor. Again, there are certain cell processes about which there can be no doubt that they consist in disintegration or breaking down, with the giving off of energy; the oxidation of glucose to carbon dioxide and water is such a case. This is dissimilation or catabolism. Now the theory of Hering is based on the idea that the two phenomena are mutually exclusive, and, no doubt, this might be the case if the molecules in question were of the same kind, as is assumed in the theory of biogens. So that if, as is pretty clear, the phenomena known especially as vital, or obvious manifestations of activity, are associated with the breaking down of molecules and giving off of energy, catabolic action will be synonymous with excitation. Further, if we accept the view that building up of new material is inconsistent with catabolic activity, we are justified in regarding the opposite process, or anabolism, as being associated with inhibition.

The theory also supposes that both these processes can be accelerated or started by nervous influences. As to the catabolic processes, there is no dispute, but we have already seen (page 288) that there is no satisfactory evidence of the direct influence of nerves on growth of protoplasm ("trophic nerves").

We have, moreover, also found evidence in various directions that material used for energy purposes does not become an integral part of the protoplasmic molecules, but is used by the protoplasmic machinery in a way analogous to that in which fuel is burnt in an internal combustion engine. The building up of a material of high potential energy, for the purpose of giving off this energy in an available form on its breakdown, appears to be effected by the concurrence of another reaction in which energy is set free by oxidation, as in the case of secretion, and we shall find a further striking instance in muscular contraction.

Again, it is very difficult to form an idea of how the increase of anabolism can result in a decrease of catabolism. Take the illustration used by Forbes (1912, 1, p. 152). The cell is compared to a water tank, provided with an inlet pipe and an outlet, both supplied with adjustable stopcocks. The outlet is supposed to be partly opened, and the stream of water represents the outgoing energy, catabolism. The inlet is connected with a supply at a somewhat higher level, and is opened to such an extent that the level in the tank is kept constant. This inflow, anabolism, is thus equal to the outflow. Now the theory under discussion implies that, if we increase anabolism by opening the inlet wider, we shall diminish the rate of outflow. In point of fact, of course, by increasing the inflow, we raise the level of

water in the tank, and this in itself *increases* the outflow owing to the rise of driving pressure. That is, increase of anabolism, or rather the anabolic state of the cell, *increases* catabolism, instead of *decreasing* it. We arrive at the same result if we regard the process from the point of view of a reversible chemical reaction. Increase of the mass of a material undergoing decomposition will increase the amount of decomposition taking place in a given time. Indeed, one cannot imagine a process in which increase of activity in one direction necessarily involves decrease in the opposite one. As Forbes puts it, to assume that increase of anabolism necessarily implies decrease of catabolism is to suppose that increasing a man's salary ensures decrease of his expenditure. To return to the tank, suppose that we reduce catabolism by narrowing the outlet, the level will rise, and consequently the inflow will diminish. That is, so far as the tank itself is concerned, the effect is the same as increasing anabolism. If we increase the outflow, we increase also the inflow. Indeed it appears as if Hering had fixed his attention too exclusively on the static condition of the protoplasm of the cell, which is certainly increased in amount by increasing anabolism. But if we look at the really important dynamic condition, there seems no doubt that increase of anabolism must also increase catabolism.

There are, on the contrary, certain facts which must not be overlooked, which appear to support this nutrition theory. If we turn to Fig. 113 (page 405) we notice that after the inhibitory pause, the first few beats are larger than those preceding the pause. It looks as if inhibition had, by increasing the contractile material, raised the functional capacity of the tissue. The question is whether this result is any greater than it would be after an equal rest produced in any other way. A further consequence of the anabolic theory would appear to be that the longer the rest, the greater the subsequent improvement. In Fig. 113 there is no relation between the two, and in Fig. 109 of Gaskell's article (1900, p. 205) the first beats are smaller than normal. We may have, in fact, after an inhibitory pause, the same condition as that shown by the "staircase" phenomenon of a ventricle which has been at rest for some time, owing to separation from the sinus.

In the case of inhibitory reflexes to skeletal muscles, we frequently find a subsequent augmentation of contraction, called by Sherrington "successive induction" or "rebound contraction." The effect of inhibition of various durations on this phenomenon has been studied by Forbes (1912, 1) and several important facts relating to inhibition in nerve centres have been brought out. There are two different phenomena concerned: the "rebound" after a brief inhibition, a contraction which is too great to be explained by mere "damming up" of "energy"; and, secondly, the effect of a prolonged period of inhibition on a subsequent excitation. It is shown that this latter effect depends on the strength of the stimulus of the inhibitory nerve. If moderate, it has a favouring effect, if strong, a depressing one; so that there is a "critical value" between the two, where no effect results. An important fact is that this critical value is lowered if the inhibitory stimulus is accompanied by an excitatory one. This result indicates that the two kinds of synapse have a more or less close relation to one another and will be found to have a bearing on the theory of inhibition. In the depressor reflex on the blood pressure in the rabbit, I found (1893, p. 320) that the state of the centre was the same before and after sixteen minutes' continuous stimulation of the inhibitory nerve, during which time the centre was in a state of inhibition, as shown by the unchanged fall of blood pressure.

The part played by *fatigue* is of interest. If a nerve which causes an excitatory reflex be stimulated, fatigue is produced after a certain time. Now it might be thought that, if an inhibitory nerve be stimulated at the same time, fatigue would be diminished. Forbes shows that the contrary is the case. Fatigue comes on *earlier* (p. 170). Further, an inhibitory reflex itself is capable of fatigue (p. 179); or rather, a prolonged inhibition, with fairly strong stimulation, diminishes the inhibitory effect of a test stimulus made immediately afterwards. To interpret this result, we must bear in mind some facts as to the seat of fatigue. As Sherrington has pointed out (1906, pp. 103-105), the seat both of excitation and of

inhibition is not in the actual motor neurone itself, but in the synapse of the afferent or intermediate neurone with it. This fact, in itself, is difficult to bring into agreement with any recognisable amount of metabolism, a conception foreign to that of a boundary surface. The motor neurones of the flexor muscles of the hind leg can be used for the scratch reflex when inhibited from being used for the ordinary flexion reflex. Of course, strictly speaking, the synaptic membrane is common to both neurones of which it forms the connecting link, but it is convenient to speak of either one without including the membrane. When fatigue of a particular reflex is brought about by stimulation of a certain afferent nerve, it is found that its motor neurones are not fatigued for a reflex brought about by stimulation of another afferent nerve. Similarly with inhibitory reflexes, we must conclude, then, that the synapses of various afferent (or intermediate) neurones with the same motor neurone are practically independent of one another. But before drawing conclusions as to the irreconcilability of fatigue with increase of anabolism, we must remember that there are, in all probability, one or more intermediate neurones between the afferent and efferent ones. And although the final synapse at the motor neurone is inhibitory, those preceding it are excitatory. Since all nerve impulses are of the same excitatory nature, that in the axis cylinder process of the intermediate neurone, which forms the inhibitory synapse with the motor neurone, is an excitation, and therefore that at the intermediate synapses is also excitatory, and it may be in these synapses that the apparent fatigue of inhibition is situated.

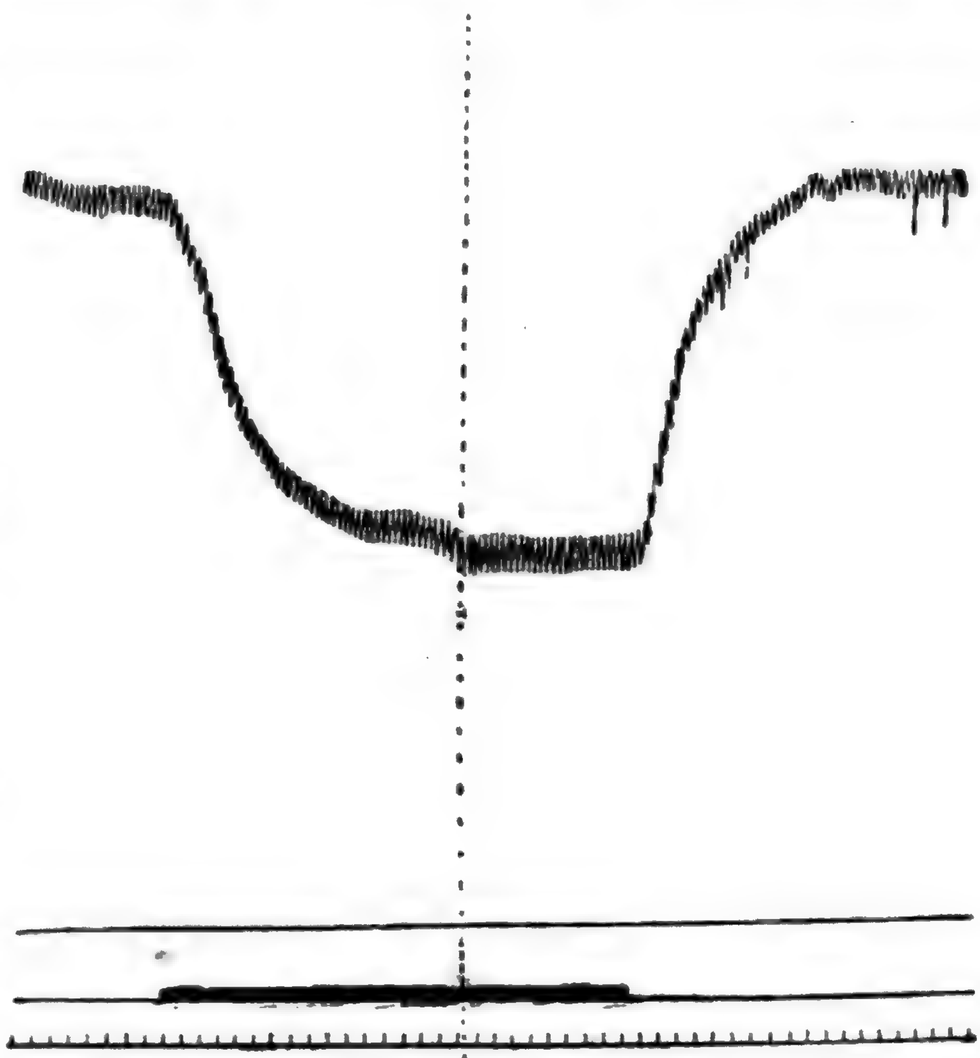


FIG. 127. TYPICAL FORM OF FALL OF BLOOD PRESSURE PRODUCED BY THE DEPRESSOR NERVE IN THE RABBIT.

The tracing shows the beginning and end of a period of stimulation lasting seventeen minutes. During the whole of this time the blood pressure remained at the level of the bottom of the curve.
Time in twelve-second intervals.

(Bayliss, 1893, Fig. 17.)

In some other cases, there is no indication of fatigue in inhibition. Gaskell (1900, p. 205) has kept the heart of the toad in complete rest for twenty-eight minutes by continuous weak stimulation of the intracranial vagus. In vasomotor reflexes, I found (1893, p. 314) that, by stimulation of the central end of the depressor nerve, the blood pressure was reduced to about half its height, and remained at this level without change for seventeen minutes; when the stimulation was stopped, the blood pressure returned to its previous level (see Fig. 127).

In the interpretation of experiments such as those of Forbes, there are two further points to be remembered. The "subsequent augmentation" might have been due to auto-stimulation by the contracting muscle of afferent fibres in its own

substance. To exclude this, the experiments were repeated on preparations in which the afferent fibres from the muscle had been cut. No difference in behaviour could be detected. The other point is that if we accept the results of various observers on "all-or-nothing" in the excitatory process, increase or decrease in the height of the reflex contraction must mean a greater or smaller number of cells in the centre in a state of activity. It would appear, therefore, that an increased response, following a period of inhibition, must be due to the inhibitory stimulus having made some synapses accessible to excitation which were previously inaccessible.

Drainage or Diversion Theories.—Another set of theories is based on the idea of a stream of excitation, flowing in a particular direction, being diverted to a different course by the presentation to it of an easier way. Apart from the "animal spirits" of Descartes, about which something will be said later, the first form in which the theory was expressed seems to be that of William James (1890, 1, p. 585, footnote). It was given more definite expression to by von Uexküll (see 1909, p. 185), who uses the names "tonus" and "excitation," which flows from one part to another of the neuro-muscular mechanisms, and by McDougall (1903), who speaks of a stream of "energy," to which he, originally, gave the name "neurin."

The simplest illustration we can take is that of a water tank feeding a fountain at a lower level than itself; there is a continual stream of water, possessing energy, passing along the pipe to the fountain. If the gardener opens a large tap on the course of this pipe, in order to fill his watering can, the fountain stops for the time, since the pressure in it falls to zero; we may say that we have "inhibited" the fountain.

Now, to begin with, I find some difficulty in discussing this view of the mechanism of the nerve centres, because the conception does not readily fit in with what we know of the nature of the excitatory process in nerve. There are, however, certain assumptions made which cannot be accepted in the form stated. It must be admitted that von Uexküll (1909, p. 58) defends himself from the imputation of using a misleading image, in speaking of a liquid flowing about in the nervous system, on the ground that the object of science is not "truth" but "order," so that we must accept his assurance that he uses such expressions as "quantity" and "pressure" of excitation and "varying capacities of reservoirs" merely to facilitate the description of experimental facts. At the same time, there appears to me to be a fundamental misconception at the base of all theories of this nature, namely, that the nerve "energy" in a given organism is a definite, limited quantity. When a part of it is diverted into some channel, it must, therefore, be drained away from some other place. But there is no reason to suppose that, when a nerve fibre divides into two, the magnitude of the propagated disturbance is diminished to half in each of the branches. Indeed, Adrian's results show that the excitation is "all-or-nothing" in both branches; since it cannot be nothing in both, it must be "all" in both. Similar conclusions are forced upon us by consideration of the electrical organ of *Malapterurus* (see Gotch and Burch, 1896, p. 387), in which the single efferent nerve fibre divides into about 1,800 branches, one to each plate of the organ. If any diminution occurred, the whole excitatory process must be frittered away to practical non-existence. If there is no diminution on branching of the nerve fibre, it is clear that adding or removing a branch will have no effect on the disturbance in the main fibre. The process is more analogous to the propagation of an explosion along a train of gunpowder, which can be made to branch as many times as desired without affecting the intensity of the explosion along the main track. In the nerve, of course, the process is reversible and perhaps unaccompanied by evolution of energy, thus differing from that of an explosion (see page 396 above).

The use of the words "stream of energy" is also inappropriate. The actual energy involved in the propagation of a nerve impulse is quite infinitesimal, as we have seen. If it be said that the words are used metaphorically, it is misleading to take a word which has a definite quantitative, mechanical meaning.

The application of the drainage theory to explain "reciprocal innervation,"

in which excitation of a particular muscle is associated with inhibition of its antagonist, is shown by McDougall (1903, p. 175) in an ingenious diagram. It shows how the two are always associated and is curiously similar to that of Descartes, which will be found described on page 495. It implies, however, that all cases of inhibition must be associated with excitation somewhere else. In our illustration, "inhibition" of the fountain is associated with "excitation" of the watering can, or of something into which the water runs. In fact, in McDougall's scheme, inhibition of a centre controlling a certain muscle can only take place by stronger excitation of the centre of its antagonist. This does not agree with experimental facts. Sherrington (1906, p. 203), moreover, objects to the theory on the ground that it makes inhibition in nerve centres a different process from peripheral inhibition of smooth muscle, heart, etc. In these latter cases, it does not seem possible to apply the drainage theory. Von Uexküll, nevertheless, does apply it, in his form, to the case of the claw of the crayfish and in the following way (1909, p. 213, and Uexküll and Gross, 1913, p. 354). There are two motor nerve tracts, ending at each muscle in a network. These networks are connected by bridges, so that any fresh excitation of either network sucks off the remaining excitation of the antagonist. In front of the network of the closing muscle there is a block of high resistance, so that weak stimuli do not reach the network. The chief evidence for the existence of such bridges seems to lie in the fact that direct stimulation of the opening muscle causes contraction in the extensor of the carpopodite, and in the presence of dividing fibres in the trunk of the nerve. It will be seen that the anatomical facts of Biedermann and of Mangold do not support this view. There is no sign of anastomosis of nerve fibres to form a network, nor of fibres which could be pointed out as connecting one muscle with the other. It is much simpler to suppose that the two different kinds of fibres which enter the same muscle fibre have opposite functions on account of the difference of the way in which they end in the muscle fibre. If each fibre of the nerve divides, as seems most probable, a branch going to each muscle, it seems that "axon-reflexes" in Langley's sense (1899, p. 388) should cause excitation or inhibition of the antagonist muscle when either muscle is stimulated directly. Which of the two would occur would depend on the strength of the stimulus, as in Richet's experiments.

Hofmann's work (1914), indeed, gives us definite information on this question. The two axis cylinders, which we have seen to run together, were found, in the case of the opening muscle, to come from two separate nerve trunks, so that they could be excited each apart from the other. One is inhibitory, the other excitatory. There is no nerve network in the neighbourhood of the muscle. It was found that the axis cylinders of the excitatory nerve send branches to the muscle of the preceding joint of the appendage, so that the result of von Uexküll, described above, turns out to be an axon-reflex, as suggested. Since the two opposing muscles are innervated by the same axis cylinder, which divides, it is clear that the simplest explanation of the facts would be that the branch to one muscle is excitatory, that to the other, inhibitory, owing to the way each ends in its muscle. Thus, one of the nerve trunks is excitatory to one muscle, inhibitory to its antagonist, while the other nerve is inhibitory to the former, excitatory to the latter. The mechanism is peripheral, instead of central, as in the vertebrate.

Block.—It might be thought that a very simple way of putting an end to the excitatory impulses playing on a nerve cell would be to make some synapse in their course impervious to excitation. Sherrington (1906, pp. 100-103) holds that inhibition involves more than this. In the decerebrate cat, the extensors of the knee can be put into contraction by a slight pinch of the opposite foot. The discharge continues for some time, gradually passing off. But if the central end of a branch of the hamstring nerve of the same leg be stimulated for a quarter of a second, the after-discharge is suddenly and completely inhibited. It seems that the efferent neurone is put into a state of excitation which continues *after* the exciting impulse has ceased, and that this state of excitation can be quelled by inhibitory nerves, although there is no exciting impulse to be blocked off.

In view of Sherrington's later work on "Plastic Tonus," to be described in Chapter XVIII., it might be suggested that this after-discharge, being itself reflex from receptors in the muscle itself, is subject to the same process as excitation from other sources. But the experiments of Forbes (1912, 1, p. 182) show that a certain amount of after-discharge is still present when

the afferent fibres from the muscle have been divided. It appears, then, that there is direct evidence that the process of inhibition implies more than the mere cutting off of impulses.

In a somewhat different sense, however, inhibition may perhaps be regarded as a kind of block. We have found reason to look upon an *increase* of permeability of a membrane as an intimate part of the excitatory process, and inhibition, as the opposite process, would thus be associated with *decrease* of permeability. But this is obviously quite a different matter from blocking the propagated disturbance itself.

Other Contributions to the Theory of Inhibition.—Although the protoplasm of the neurone is probably a liquid, it contains various substances in the colloidal state. Further, we have seen the necessity of the presence of electrolytes to account for the electrical changes in nerve Now, if these electrolytes lower surface energy, they will be adsorbed on the surface of the colloidal particles (see page 55). This is the foundation of the *theory of Macdonald* (1905) as to the nature of excitation and inhibition. This investigator points out (p. 335) how the concentration of electrolytes in the external phase would be increased if anything in the nature of aggregation or coagulation in the nerve colloids occurred. The adsorbing surface would be diminished. Assuming that these electrolytes are essential to excitation, it will be seen how a coagulation process would be associated with excitation, while a greater dispersion than normal would be associated with greater adsorption of electrolytes and inhibition (Macdonald, p. 348). This is a brief account of this important theory. Details of its application require more knowledge than we possess as yet of the phenomena taking place at the membranes. If we place the seat of excitation and of inhibition of nerve cells at the synaptic membrane, we may suppose that the system of colloids and electrolytes in question either forms the membrane itself or is intimately associated with it.

In discussing Hill's modified form of Nernst's equation for excitation, we saw (page 394) that it contains a constant, C, which is connected in some way with adsorption (or disappearance) of ions. Keith Lucas (1910, p. 243) shows that it is altered by removal of calcium. It seems not unlikely that changes in the value of this factor, C, might be made use of to investigate the hypothesis of Macdonald.

With regard to the properties of the *synaptic membrane*, Keith Lucas (1911) points out that it must present a greater resistance to conduction than the axis cylinder does; it is thus similar to the junction between nerve and muscle or to a narcotised region in nerve. There is thus a possibility that it might obliterate very rapid, and therefore small (see Wedensky inhibition, page 420), nerve impulses, so that they would not get through the synapse. Adrian (1912, p. 411) calls attention to the fact that an impulse, if it has been able to pass a region of decrement at all, recovers its full size on arriving in normal nerve again; so that, in order that the block mechanism above referred to may be effective, the impulses must be reduced to zero in one of the synapses; unless this were the case, it would not matter how many separate regions of decrement the impulse had to traverse.

It cannot be said that any one of the theories suggested is a satisfactory one. Perhaps each, when modified in accordance with certain important parts of the others, has a part of the truth, and there may be particular fields in which aspects of one theory have more part to play than in other fields.

There are certain facts which admit of no doubt, and any theory must reckon with these. The function of any particular nerve fibre depends on its termination; the nature of this termination determines whether it excites or inhibits. In the case of smooth muscle or heart and certain nerve centres, which have an inherent state of excitation, each element requires two nerve fibres to modify its state, increase it or decrease it. In Langley's view, each of these nerve fibres ends in a distinct receptive substance, whose qualities determine whether the effect is excitatory or inhibitory. It appears also that, of the various nerve fibres forming synapses with a particular nerve cell, each has its definite character of inhibiton or of excitation. In such cases as those of reciprocal innervation, Sherrington points out (1906, p. 105) that the inference must be made that each afferent nerve fibre concerned in the reflex divides into two parts in the spinal cord, one set of

these subdivisions being excitatory, the other inhibitory, in respect of the motor neurones. This is similar to the most probable arrangement in the crayfish claw, namely, that each nerve fibre divides into two, one division going to the closing muscle, the other to the opening muscle; the two parts into which each fibre divides are always one excitatory, the other inhibitory.

Michailov (1911) describes two different kinds of nerve endings in the muscular tissue of the heart. He regards one of them as sensory, the other as the inhibitory endings of the vagus. It is, of course, also possible that the former might be the terminations of the sympathetic supply.

We have found evidence that the membranes which are the seat of the excitatory process are of such a nature as to be impermeable to one only of the two oppositely charged ions into which certain electrolytes in the nerve are dissociated. It may possibly happen that, when the impermeable ion is allowed passage by the arrival of the propagated disturbance, it produces chemical or physical changes in the substance of the inhibitory synapse of such a kind as to render excitatory synapses on the same cell incapable of undergoing the normal change of permeability associated with the passage of an excitation. In the present state of knowledge, it is unprofitable to follow such speculations far, and the suggestion is made merely as an indication of a possible mode of explanation.

REVERSAL EFFECTS

Although under ordinary conditions, such as change of intensity of stimulus, altered time course, and so on, an inhibitory termination of an afferent arc cannot be made to give any other effect than inhibition, nor an excitatory termination anything but excitation, there is evidence, as Sherrington points out (1906, p. 105), that under special conditions an inhibitory termination can be made into an excitatory one, and vice versa. Certain drugs have effects of this kind. Moreover, from the cerebral cortex, antagonistic muscles can be put into action at the same time. We saw above that stimulation of the central ends of various afferent nerves of the hind leg produces inhibition of the contraction of the vasto-crureus in the decerebrate cat. Now, Sherrington found (1905, p. 287) that a small dose of strychnine converts this effect into a reflex *contraction*. I found the same fact in the case of the inhibition of the vaso-constrictor centre by the depressor nerve (1908, 2), and Seeman (1910) in respiratory reflexes. Sherrington also showed that tetanus toxin does the same. It is to be noted that the vasto-crureus is a purely extensor muscle and can be completely isolated. By careful gradation of dose, a stage can be obtained in which the inhibitory effect is diminished, but not replaced by excitation. This fact seems to show that the result is not to be explained by decreased resistance allowing the stimulation to spread to other neurones in the centre, since the resistance at this stage is increased. Another possibility is that the afferent nerve stimulated might contain, along with the fibres causing inhibition, others causing contraction, and that the effect of strychnine might be to paralyse the inhibitory reflex fibres or synapses before the excitatory ones. Now these inhibitory fibres would be associated with those causing contraction of the flexors and no trace of depression in the force of the flexor contractions is to be detected. The antagonist muscles are simultaneously thrown into contraction. I have shown, moreover (1908, 2), that the strychnine reversal of the inhibition of the vasomotor centre is not due to unmasking of excitatory fibres mixed with the inhibitory ones, by paralysis of the latter. The dose of the drug required in the rabbit is a comparatively large one and, at the time that the reversal is most perfect, it is found that the excitatory vaso-constrictor fibres in other afferent nerves are completely paralysed, so that the usual rise of pressure from ordinary sensory nerves is absent. It seems on the whole that the view taken by Sherrington at the first (1906, p. 111) is the most probable one, namely, that the action of strychnine "is to convert in the spinal cord the process of inhibition—whatever that may essentially be—into the process of excitation—whatever that may essentially be. The reflex nexus was pre-existent, but the effect across it was signalised by a different sign, namely, *minus* prior to the strychnine or tetanus toxin, instead of *plus*, as afterwards."

In a later paper by Sherrington and Owen (1911) the impossibility of a definite decision is pointed out until we have a nerve which can be proved to contain no afferent excitatory fibres. Strychnine also reverses the inhibitory component of reciprocal innervation in the starfish and in *Planaria* (A. R. Moore, 1919, 1) and in the earthworm (Knowlton and Moore, 1917). Cushny (1919) holds that reflex strychnine tetanus is due to an increase of the "start" reflex.

Chloroform is well known to be, pharmacologically, a general antagonist to strychnine; it is not unexpected, therefore, to find that its action on nerve processes is the reverse of that of strychnine. I showed (1908, 2) that it converts the excitatory component of a vasomotor reflex into an inhibitory one. That is, the rise of pressure produced by a nerve which excites the vaso-constrictor centre is converted into a fall. Examples of this fact are given in Fig. 128, from a paper by Mathison (1912), and Fig. 129 from one of mine. Similarly, Sherrington and Sowton (1911, 2) showed that the reflex contraction of the vasto-crureus, evoked by stimulation of the popliteal, internal saphenous or genito-crural nerve, was converted into inhibition by the administration of chloroform.

Reversals of this kind can be brought about without the influence of drugs, as shown more particularly by Magnus (1909). The direction of the movement of the tail of a spinal cat, when the tip is pinched, varies with the position in which it hangs. The movement is always towards the stretched side, so that the same afferent impulse produces, in one position, excitation of those muscles which are inhibited in the opposite position. The condition of the centre must be changed by receipt of afferent impulses from the stretched muscle.

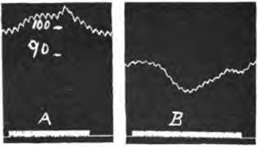


FIG. 128. REVERSAL OF VASO-CONSTRICTOR REFLEX BY CHLOROFORM.

Stimulation of central end of hypoglossal nerve in the rabbit.
Left-hand tracing—under urethane alone.
Right-hand tracing—under 3 per cent. chloroform in addition.

(Mathison, 1912, Fig. 2.)

(By the kindness of Dr Ninian Bruce.)

Returning to the action of chemical agents, Dale, Laidlaw, and Symons (1910) describe how stimulation of the vagus in the cat, under the effect of nicotine, causes marked acceleration instead of slowing of the heart. This may be due to earlier paralysis of the inhibitory fibres than of supposed accelerator fibres, normally masked. Or it may be a reversal of the function of the inhibitory fibres. In the latter case, we should have an instance of reversal of a peripheral muscular mechanism. The authors named are inclined to favour the former hypothesis, but it does not seem probable, *à priori*, that fibres acting in the same way as the sympathetic supply of the heart should be present in a cranial nerve.

Again, Dale and Laidlaw (1911) found that, after a dose of cytisine, the alkaloid of laburnum seeds, stimulation of the chorda tympani nerve in the cat produces no secretion while the stimulus lasts, but is followed by a copious flow. A renewed stimulation nearly stops, temporarily, this after-flow.

The authors suggest no explanation, but it seems to me, although I admit that the suggestion is purely hypothetical, that, if we regard the action of strychnine and of chloroform as actually reversing the sign of the effect on a nerve cell, why should we not accept the possibility of similar reversal in peripheral inhibition and excitation in smooth muscle and heart? If this be so, the above result on the salivary secretion might easily be explained by conversion of the normal vaso-dilator action of the chorda tympani into a vaso-constrictor one, the diminution of flow being then due to failure of blood supply. This hypothesis might be tested by determining the rate of blood flow under the conditions of the experiment.

Langley (1911) finds that, after nicotine or curare, the normal contraction of the bladder produced by stimulation of the sacral nerves is followed by inhibition,

and that the presence of inhibitory fibres is not a satisfactory explanation. Changes in the sign of the movement of ions to or from the membrane is suggested, a process which would be equivalent to reversal of excitation into inhibition.

The fact, discovered by Dale (1906), that ergotoxin converts the normal vaso-constrictor effect of the abdominal sympathetic nerve on the hind leg into a vaso-dilator one is, to my mind, more consistently explained by peripheral reversal than by paralysis of vaso-constrictors leaving unmasked vaso-dilator fibres. I have been quite unable to find any evidence of the existence of such fibres in the abdominal sympathetic chain.

Spaeth and Barbour (1917) have shown that adrenaline produces contraction of the melanophores of a fish, Fundulus. This fact indicates that they are modified smooth muscle cells and under sympathetic control. Previous treatment with ergotoxin not only abolishes this contractile action of adrenaline, but reverses it to an expansion. The interest lies in the action being visible on a single cell.

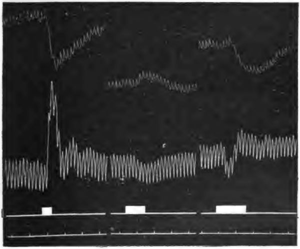


Fig. 129. THREE STIMULATIONS OF THE CENTRAL END OF THE MEDIAN NERVE IN THE RABBIT.

Upper tracing—volume of the kidney.

Lower tracing—arterial pressure. Zero is 23 mm. below the upper signal.

The first stimulation is under ether alone. There is a rise of blood pressure, with vaso-constriction in the kidney.

The second is under chloroform. There is a *fall* of blood pressure, with vaso-dilatation in the kidney.

Third stimulation after partial recovery from chloroform under ether. Preliminary vaso-dilatation, followed by a larger vaso-constriction.

(Bayliss, 1908, 2, Fig. 24.)

But the whole question as to the mechanism of these various reversal phenomena cannot be said to be capable of decision as yet.

Pearce (1913) has described experiments in which the normal action of adrenaline on the arterioles of the frog appeared to be converted into a dilatation in the absence of calcium. I have been unable to confirm this result. Adrenaline produces its normal effect on the auricle when the calcium of the perfusion fluid is reduced as far as possible without cessation of the beats.

EXCITABILITY IN PLANTS

The mechanism by which movements are produced in plants, especially in the higher plants, will be discussed in the next chapter. The nature of the excitation process concerns us here.

That it is essentially similar to that in the animal is indicated by the fact that vegetable protoplasm, in a state of excitation, is electrically negative to that at rest. This is shown by the observations of Hörmann on *Nitella* (1898, pp. 69-79). A stimulus at a point sets a wave of excitation in progress, accompanied by a state of electrical negativity. This electrical state, so far as could be ascertained, precedes the cessation of movement caused by the stimulus. In these cells, the streaming movement corresponds to the contractile properties of a muscle fibre and is, as we have seen, something added on to the simple excitatory process as it shows itself in nerve. Similar conclusions are to be drawn from the work of Burdon-Sanderson on the leaf of *Dionæa muscipula* (1882 and 1888), whose leaves shut up rapidly when a fly touches certain sensitive hairs on the upper surface. By placing electrodes on opposite lobes, and stimulating the neighbourhood of each electrode in turn, it was shown that the excited spot becomes first negative, then positive, as the wave of excitation reaches the other electrode (see Fig. 130); just as was described above for nerve and muscle. It is pointed out that this wave of excitation precedes the change of form and travels at a much faster rate. Visible change of form was prevented by a cross-bar fixed between the lobes.

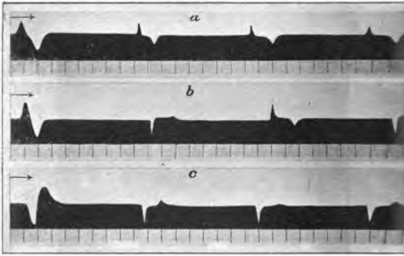


FIG. 130. ELECTRICAL CHANGES IN THE LEAF OF DIONÆA.—Corresponding points on the under surface of each lobe led off to capillary electrometer.

a, Stimulated mechanically four times on right side. Diphasic effect due to excitatory process arriving at each electrode in turn.
b, Stimulated on right and left sides, alternately.
c, Stimulated on left side, four times.

Time signal—twenty per second.

(Burdon-Sanderson, 1888, Figs. 8, 9, and 10.)

It is a familiar fact that plants in general, root, stem, leaves, and flowers, respond to *gravity and to light*, and in various ways. Certain of them, as the climbing plants, and especially the "sensitive plant," and *Dionæa*, respond to *touch* by more or less rapid movements.

Now this response is not confined to the part actually stimulated, but the excitation is conducted to more distant parts. For a description of the various phenomena concerned, the reader is referred to the book of Pringsheim (1912). We are chiefly interested here in the excitatory process itself.

It is found that a stimulus, such as gravity or light, requires to act for not less than a certain time in order to have any effect at all. This minimum time is known as the "presentation time." Further, however long a stimulus is applied, no effect is produced until an interval of time has elapsed since the beginning of the stimulation. This is the "reaction time."

There are no special channels for conduction of excitatory processes like the nerves of animal organisms. Conduction appears to take place through the cell protoplasm, which must be living. In the stem of *Tradescantia virginica*, the curvature which takes place under the stimulus of gravity takes effect on the next internode below the one stimulated, and the transmission is abolished by local anæsthesia of a spot between the two. Similarly, light stimulus acting on the stem of the Dahlia is transmitted to the root. A curious fact, whose probable explanation will be given in Chapter XVII. on receptor organs, is that, at the temperature of 0° or in an atmosphere of hydrogen or carbon dioxide, no effect is produced until the temperature is raised or oxygen supplied, respectively. But, although the actual stimulus may have ceased before the change of conditions, the effect shows itself

When light stimulus and gravity stimulus act together, the former, as a rule, completely overpowers the latter.

The manner of conduction has been a subject of dispute. In the case of the sensitive plant, it was originally held by Pfeffer and by Haberlandt that the transmission was mechanical, by a movement of water in tubes of the vascular bundles; but the abolition of power of conduction by local anæsthesia is strong evidence that it takes place through protoplasmic structures. It is known in many cases that the protoplasm of neighbouring plant cells is united by strands passing through holes in the cell walls (see especially the work of Gardiner, 1884). Fig. 131 shows the structure of a tissue of this kind. Further details as to the mechanism of conduction will be found in the essay by Fitting (1906).

FIG. 131. SIEVE TUBE.

The protoplasts, contracted by the action of alcohol, adhere to the transverse wall, and that of each cell is connected to the other by delicate protoplasmic filaments, passing through the pores of the cell wall.

Magnified.

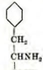
(From Timiriazeff.)

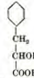
In connection with the increase of permeability which we have seen to occur in the state of excitation, the mechanism of the movements of the sensitive plant, investigated by Pfeffer (1873), is of interest. As we have seen, the vegetable cell is maintained in a state of turgor by means of the osmotic pressure due to the presence within it of substances in solution, and to the impermeability of the cell membrane to these solutes. Since the cell wall surrounding each is incapable of any considerable stretching, a pressure in the interior results. A mass of cells with such a turgor well developed exists at the lower side of each movable joint in the leaf of the plant. The cells on the upper side are less turgid. When stimulated, the cell membrane of the lower cells suddenly loses its semipermeable character, as regards the solutes of the cell contents, with the consequence that the internal pressure can release itself by filtering solution through the membrane. Drops appear on a cut surface and the weight of the leaf, being no longer supported by the distended cells, causes it to fall.

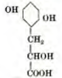
V. H. Blackman and Paine (1918) hold that the loss of turgor is due to a decrease in the osmotic pressure of the cell constituents, rather than to their actual escape, which is not great enough to account for the effect.

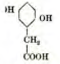
Another phenomenon, which may be in some way connected with changes of permeability, is the oxidation reaction, described by Czapek and Bertel (1906). If longitudinal sections are cut from the root point of lupin seedlings, it is found that their cells become brown on boiling with ammoniacal silver nitrate, owing to the presence of a reducing substance. If the root has been stimulated geotropically, the dark stain is more intense.

This was due to homogentisic acid, produced from tyrosine. But the OH-group in the latter is in the para-position, in the former in the meta-position. If, however, the OH be first removed from tyrosine, phenyl-alanine is formed, and from this, by de-amination and oxidation, homogentisic acid might be produced thus:—


Phenyl-alanine.


Phenyl-a-oxypropionic acid.


Uroleucic acid.


Homogentisic acid.

After the action of gravity, then, there is more homogentisic acid found in the root than normally. This is interpreted as follows: homogentisic acid is oxidised to carbon dioxide and water in the resting cells; under the action of gravity, an "anti-oxidase" is formed, which inhibits the normal oxidation process. Since, as we have seen, the existence of specific anti-enzymes is extremely doubtful, it would be more correct to say that the action of gravity caused the appearance of some substance which retards the action of the oxidase. A similar effect was produced by heliotropic stimulation. Prevention of movement does not alter the reduction, so that this is a result of excitation as such.

Homogentisic acid plays a part in the normal oxidation of tyrosine in the animal organism (see Garrod, 1909, pp. 41-81), since, in certain inborn errors of metabolism, the enzyme responsible for the further oxidation of homogentisic acid is absent, and administration of tyrosine increases the output of homogentisic acid. In Garrod's book (p. 78) another mode of conversion of tyrosine into homogentisic acid, through para-oxyphenyl-pyruvic acid, is given.

SUMMARY

Automatic cell processes require the provision of means of regulation in two directions, increase and decrease. The former is called "excitation" and the latter, "inhibition." A process which is set into action by influence from without may also be stopped by inhibition of the external influence.

Processes of a non-living nature are also capable of modification in two directions by external action, as in the familiar case of reversible reactions.

Strictly speaking, all living protoplasm is able to respond to external changes ("stimuli"), but the name of "excitable" tissues is given for convenience to those which, like muscle and nerve, respond by rapid changes.

Nerves are especially present for the purpose of conducting a stimulus from the place of application to more distant parts of the organism and bringing the various constituent parts into relation with one another. They really constitute *the* excitable tissue, *par excellence*, since they have no other function to perform.

A nerve when disturbed at a point conveys some sort of change, the propagated disturbance or nerve impulse, along its course to the place where the nerve fibres terminate, and the tissue in which the fibre ends is excited or inhibited according to the particular manner in which the fibre is connected. Of course, the kind of activity which is set going or stopped depends on the cell, nerve cell, muscle fibre or gland cell, etc.

There is no visible change in a nerve as the impulse traverses it. Indeed, one kind of change only has been definitely shown to take place, an electrical one, in which a spot in activity is electro-negative to one at rest. Certain conclusions as to the nature of the process can be drawn from this fact, taken in connection with

the way in which the nerve responds to stimulation. There is no heat produced, and the evolution of carbon dioxide is questionable.

The various practical methods of setting up a propagated disturbance in excitable tissues are described in the text.

There are no differences of degree in the state of excitation of a nerve or muscle cell in a given state; a stimulus either produces the maximal effect that the tissue is capable of in this condition, or no response at all, in the way of a propagated disturbance. Nevertheless, a stimulus too weak to do this leaves behind a local change at the point of application. Degrees of contraction, produced in a muscle by different intensities of stimulation, are due to the activity of a larger or smaller number of fibres in the nerve or muscle.

A narcotised region of nerve reduces the intensity of a propagated disturbance as it passes along it, so that, if long enough or sufficiently deeply narcotised, it abolishes the disturbance altogether. But, if any state of excitation is left at all, the disturbance returns to its original magnitude when it enters a normal region again.

It appears, then, that the degree of activity of a tissue supplied by nerves depends only on the number of tissue cells acted upon, and on the state of these particular cells; not on any difference of degree in the stimuli reaching a given cell. It should be mentioned that this view is not accepted by all investigators so far as it applies to reflexes from the central nervous system.

All excitable tissues are incapable of response to a second stimulus applied at a short interval of time, differing in different tissues, after a previous one. This is the "refractory period," and consists of a first part, where no strength of stimulus whatever will excite ("absolute refractory state"), and of a second part ("relative refractory state"), where stimuli stronger than normal are required. The disturbances set up in this latter period are smaller than normal, but cannot be made greater than those set up by a stimulus just sufficient to excite, however the stimulus is increased. Their magnitude increases progressively up to the end of the refractory period.

The refractory state is not only local, but follows the propagated disturbance as it passes along the nerve fibre.

The question of fatigue of nerve fibres is somewhat disputed. There is some evidence, not altogether convincing, that oxygen is necessary for the continuance of the excitability of a nerve fibre.

The electrical negativity associated with the passage of an impulse is followed by a state of increased positivity, the explanation of which is not yet clear.

The passage of the nerve impulse takes time, the rate being increased by rise of temperature. The temperature coefficient is 1·79 for 10° C.

There is evidence that the state of excitation is accompanied by increased permeability of the cell membrane. If the membrane be impermeable, at rest, to one only of the ions of an electrolyte within the cell, the membrane is "polarised," and the "current of rest," "injury current," or "demarcation current," is accounted for. If this semipermeability is abolished in excitation, the "negative variation" can be accounted for, and also the diminished polarisability in this state.

A certain formula was put forward by Nernst to express stimulation by an electrical current. The basis of this expression is a movement of ions to or from a semipermeable membrane and is suggested as an approximation only. Taking further known facts into consideration, A. V. Hill modified the formula in a way which was found by Keith Lucas to satisfy most cases of experimental test. The factors playing a part are the number of ions and their charge, the distance between the membranes, and the distance of the place where the concentration takes place from the membrane under consideration, the rate of movement of the ions, and a factor expressing rate of "recombination" of ions. This last factor is probably an adsorption in the sense of Macdonald's theory.

Various excitable tissues differ in the rate of incidence of their "optimal" stimuli, that is, the stimulus which excites with the least expenditure of energy. This is Waller's "characteristic," and is included in the modified Nernst formula.

The function of the medullary sheath is still problematical, although it appears to have a function in connection with the growth of fibres. The axis cylinder is probably of a liquid nature, but colloidal. There is no evidence of the existence of "neuro-fibrils" in the living state.

The nerve impulse, as it travels along a fibre, seems to be a reversible, physicochemical process, not associated with metabolic changes; but the question is not, as yet, altogether decided.

A distinction must be made between the local process at the spot stimulated and the propagated change. The former is confined to the stimulated spot, and requires a certain small expenditure of energy to set it up. When set up, if sufficiently intense, it produces a propagated disturbance. There is no convincing evidence that the latter is attended with any consumption or evolution of energy.

In muscle there is an excitatory process essentially like that in nerve, and, superadded to this, a contractile process, which has a latent period and is associated with metabolism, with its accompanying heat production and fatigue. The former process may be present without visible contraction.

There are certain other excitable substances which intervene between nerve and muscle. These are called "receptive substances" or the "myo-neural junction." Their existence can be shown by their reaction to various drugs, and by their different optimal rates of stimulation. The muscle fibre has a low optimal rate, the nerve trunk one of a rather higher value, while the intermediate substance has a very high one. These optimal rates have been shown by Keith Lucas to be functions of the rate of diffusion of the ions concerned in the excitation process, according to the conception of Nernst.

There is a membrane intervening between the nerve ending and the muscle fibre supplied by it, as also between one neurone and the fibre connecting it with another neurone. This membrane is called by Sherrington the "synaptic membrane."

The essential processes connected with inhibition must be of an opposite kind to those connected with excitation. Various theories have been propounded, from different points of view, as to what is the basis of inhibition.

In the text, a number of illustrations are given to show the different aspects of inhibition, as it affects peripheral muscular organs directly, and as it affects nerve centres acting on these organs through nerves. Also as it affects the nerve centres controlling those peripheral organs, such as skeletal muscle, which have no automatic activity of their own.

That the excitatory and inhibitory influences act on the same cell is shown, amongst other facts, by the capability we have of exactly neutralising the effect of stimulation of the one nerve by simultaneous stimulation of the one having the opposite effect.

The state of excitation of a nerve centre causing peripheral inhibition can be itself inhibited by nerves acting on this centre, so that we have "inhibition of inhibition." It seems probable that an intermediate neurone, inhibiting a particular motor neurone, may itself be inhibited by an afferent neurone and thus the inhibition of the motor centre may be taken off.

Higher centres in the nervous system influence lower centres, either increasing or decreasing their excitability. Hence the phenomena of "spinal shock" and of "decerebrate rigidity."

Inhibitory effects can be brought about by direct action of chemical agents or by the anode of the electrical current.

The phenomena of physical interference of wave motion are incapable of

explaining total inhibition, whereas it is an experimental fact that inhibition may be complete.

The part played by the refractory period is described in the text. Also an explanation is suggested for the results of von Frey on the vasomotor nerves of the submaxillary gland.

It is shown how the theoretical basis of the theories according to which "assimilation" or "anabolism" is associated with inhibition is unsatisfactory, and that, if we look upon cell processes from the dynamic point of view, increase of anabolism seems to necessitate increase of catabolism also, instead of decrease, as such theories of inhibition require.

There is no satisfactory evidence that the increase of functional capacity, some times found to be present after inhibition, is actually due to an increased building up by the inhibitory stimuli. On the other hand, the fact that fatigue of inhibition in nerve centres is found to occur does not necessarily exclude an anabolic explanation, since the fatigue may be situated in an intermediate excitatory synapse.

The seat both of excitation and of inhibition in nerve centres is in the synapses, so that fatigue must in all probability be here also.

There is a fundamental misconception at the basis of the "drainage" theories, namely, that the amount of nerve "energy" present in the neuro-muscular system is a constant, limited quantity. Moreover, the occurrence of inhibition without simultaneous excitation elsewhere is left unexplained by such theories.

The possibility of production of a "block" and its relation to the permeability changes of the membrane is briefly discussed.

Macdonald's theory of adsorption of ions by colloids is shown to have considerable importance as a contribution to the theory of inhibition and excitation.

Inhibition can be changed into excitation by strychnine and similar drugs, while excitation can be changed into inhibition by chloroform. The most satisfactory explanation seems to be that the actual processes themselves are reversed. The possibility of a similar phenomenon being concerned in the reversal of peripheral action, such as that of the vagus and of the chorda tympani by certain alkaloids, is pointed out.

The process of excitation in plants, apart from movement, is shown to be essentially similar to that in animals, being independent of visible effects, and accompanied by electrical negativity of the excited protoplasmic structures.

Conduction of excitation in plants appears to be through protoplasmic continuity of cells, since it can be abolished by local application of anæsthetics.

The "anti-oxydase" reaction of Czapek, as associated with stimulation in plants, is described.

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Excitation in Plants.

Pringsheim (1912). Burdon-Sanderson (1888).

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Sherrington (1906, pp. 83-106, 133-148, 191-199). Macdonald (1905, pp. 331-350).
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CHAPTER XIV

CONTRACTILE TISSUES

In the animal organism, the tissues which have the power of effecting movement by changing their form, "contraction," as it is usually called, are known as muscular. It should be made clear at the outset that the word "contraction" is, strictly speaking, incorrect, since there is no change in volume when a muscle becomes active, merely change of shape, by which its two ends are brought closer together. So that, if one end is fixed, the other end moves nearer to it and, if the latter is attached to a movable object, this object moves with it. If the muscle is prevented from shortening, owing to attachment to an immovable object, a state of tension is developed in it.

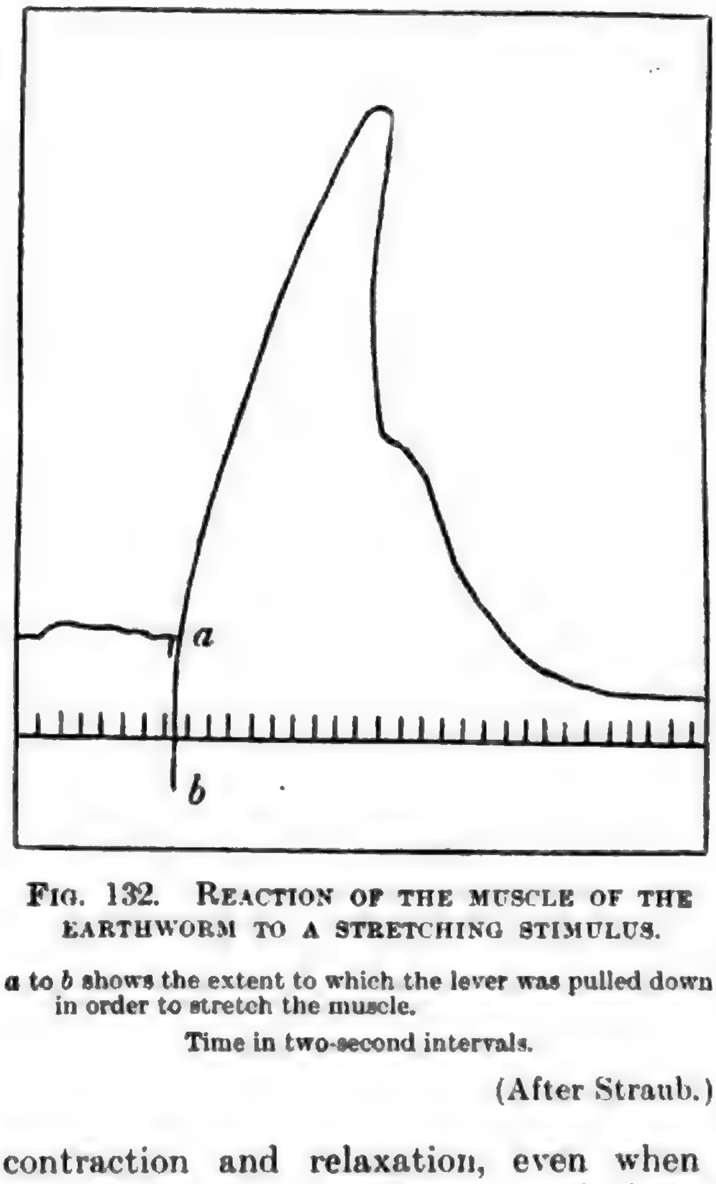

Fig. 132. Reaction of the muscle of the earthworm to a stretching stimulus.

a to *b* shows the extent to which the lever was pulled down in order to stretch the muscle.

Time in two-second intervals.

(After Straub.)

The mechanism of movement in the plant is of a different kind, and will be described in a special section later.

There are two kinds of muscular tissue, which, in the extremes of the scale, have very distinct properties, namely, the cross-striated, skeletal, or voluntary muscle on the one hand, and the smooth, non-striated, or involuntary muscle on the other hand. There are, however, many degrees of transition between them. The heart muscle of the vertebrate is cross-striated, but exhibits many of the properties of the other class; the claw muscle of the crayfish is another case.

Perhaps the most characteristic difference, physiologically, between the two classes is that the typical skeletal, cross-striated muscle, in its highest form of development, is entirely dependent on impulses from the central nervous system to set it into activity; the other class possesses an automatic activity, manifested in tone, or in rhythmical contraction and relaxation, even when separated from the central nervous system. It is not to be supposed that the involuntary muscle is not subject to control from the central nervous system; we have seen the contrary to be the case with the intestine, the heart, the blood vessels in the vertebrate and the claw in the crayfish. This last case is, indeed, a difficult one to classify on any system, since, although possessing automatic tone, it is under voluntary control on the part of the animal, like the skeletal muscles of the vertebrate. The organs consisting of smooth muscle in the latter organisms, and also the heart, are not under voluntary control, although acted on reflexly.

Some other differences, rather of degree than of kind, may be mentioned. The rate of contraction of the smooth muscle is usually slow, compared with that

of striated muscle; it has also a longer latent period. There are two properties deserving mention which both classes of muscle possess, although produced in a different way. The automatic *tone* of smooth muscle has been referred to above; skeletal muscle, under normal conditions, also possesses a certain degree of tone, but it is of reflex origin from afferent nerves in the muscle itself and the joints, etc., ceasing when the nerves are cut. Again, smooth muscle is caused to enter into contraction by ***stretching***, as shown in Fig. 132 (from Straub's paper, 1900). The possible importance of this reaction to stretching will be discussed under vasomotor mechanisms. The changes in the tonus of skeletal muscle produced reflexly by changes of position of the ends of the muscle (that is, changes in the length of the fibres) were investigated by Sherrington and will be described under the head of "plastic tonus" in Chapter XVIII.

STRUCTURE

Details of structure, especially in the case of the complex one of cross-striated muscle, are extremely difficult to make out at all satisfactorily. Very little, except the alternate dark and light bands, can be seen in living muscle, and we have no guarantee that the various structures seen by different observers, after treatment with reagents, have any resemblance to the living state. The account given by Macdonald (1909) will be read with profit by those interested in the views that have been put forward.

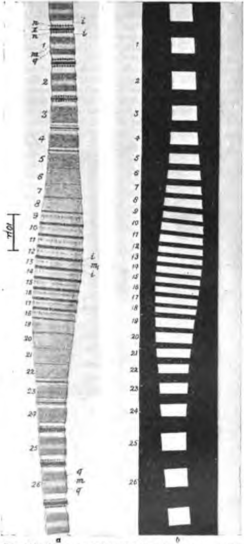

FIG. 133. MUSCLE FIBRE WITH CONTRACTION WAVE.—From abdomen of *Telephorus melanurus*. Fixed by 50 per cent. alcohol.

a, in ordinary light. *b*, in polarised light, between crossed Nicols.

The wave length is about 25 striæ; the maximum degree of shortening is about 75 per cent., and is at Nos. 12, 13, and 14 of the figure. Nos. 8-19 show in *a* reversed position of dark and light bands, but this reversal is absent from *b*.

Note also the intermediate stage of Nos. 5-7 and 20-22, in which the cross-striation in *a* nearly disappears.

In *b* it is seen that, in contraction, the volume of the anisotropic part (white) increases at the expense of the isotropic part (black). The figure should have represented the ratio of the heights as 3 : 1.

i, Isotropic disc. *n*, Accessory disc. *z*, Intermediate disc. *m*, Middle disc. *q*, Transverse disc. (After Engelmann.)

It seems possible that photographs of the living fibre by ultra-violet light, in the manner described in a previous chapter (page 9), might give valuable information with regard to the structure of muscle.

A fact of interest is that the dark bands of striated muscle are doubly refracting, that is, they appear bright on the dark field between crossed Nicol prisms. If a fibre is made to contract while under the microscope, the dark band becomes light and the light band dark; but the double refraction does not alter, that is, it is the light band which is now doubly refracting. According to Engelmann (1873, p. 166), the isotropic (singly refracting) part diminishes in volume in contraction, while the anisotropic (doubly refracting) part increases; that is, fluid passes out of the isotropic into the anisotropic elements (p. 167 of the paper). Fig. 133 shows this and other facts. It will be seen, in the appearance under polarised light, that whereas in the resting fibre the two parts are of about equal size, in the contracted part, in the middle of the portion of fibre represented, the clear part considerably exceeds in volume the dark part. Further, Engelmann holds (1875) that contractility is always associated with double refraction; in the striated muscle fibre it is the anisotropic part which is the active constituent, and Engelmann has detected double refraction in the contractile parts of *Hydra*, and of various unicellular organisms. The statement is made (p. 460) that contractility, in whatever form it may occur, is connected with the presence of doubly refracting, positive, uniaxial particles, whose optical axis coincides with the direction of shortening. The isotropic part is supposed capable of excitation, but not of contraction.

Hürthle (1909) concludes, from the evidence of photographs of living fibres, that no change of volume occurs in the doubly refractive, contractile elements. The isotropic parts, on the contrary, increase in volume at the expense of the sarcoplasm. Although the photographs of the living fibres with waves of contraction are certainly very interesting, it is difficult to make out from them whether the statement is justified (see Schäfer's criticism of Hürthle's views, 1910, pp. 72-73). Fig. 134 shows photographs of living muscular fibres under polarised light.

FIG. 134. FIBRE OF LEG MUSCLE OF CHRYSOMELA CŒRULEA WITH (FIXED) CONTRACTION WAVE, PHOTOGRAPHED UNDER POLARISING MICROSCOPE.

A, with parallel Nicols. *B*, with crossed Nicols.

(After Engelmann. Schäfer's "Essentials of Histology," Fig. 166, p. 136.)

DEVELOPMENT OF TENSION

The essential point in the mechanics of muscular contraction is that the properties of the tissue change in contraction, so that, if it be not permitted to shorten, a state of tension is developed. It is a difficult matter to give an adequate mechanical illustration of the process, but perhaps it will assist comprehension if we imagine that we have two spirals, one of hardened steel wire, the other of soft lead wire. We now stretch them to the same extent. The coil of steel wire will be in a state of tension, as felt by the necessity to exert a continuous pull upon it to prevent its returning to its original length, whereas the lead wire will have

no tension, oppose no resistance to stretching, and will not return to its original length when released. When a muscle is stimulated, it changes its physical state from that of the *stretched* coil of lead wire to that of the *stretched* coil of steel wire. The fact may also be realised if we allow a muscle to shorten on stimulation and then pull it back to its original length, the stimulation being continued. It will require a certain force to do so; this force may be measured by the weight which it is necessary to hang on the end of a vertical muscle in order to bring it to its resting length. This is, of course, only a rough measurement, because the weight will passively stretch both the resting and the contracted muscle; so that it will be found that if the weight is applied which is just sufficient to extend the muscle to its unloaded resting length, the weight will fall somewhat when the stimulus ceases, since it stretches the resting muscle to a greater length than its unloaded one. This is the fact involved in the principle of "*after-load*," in which the weight is supported at the position of the length of the resting muscle and does not stretch it until contraction takes place.

As we shall see in considering the heat evolved and also in the theory of muscular contraction, the development of tension is the fundamental fact in the process, so that it is of importance to grasp its meaning clearly.

The special mechanism by which muscles are able to possess the same degree of tension at different lengths requires separate consideration and will be discussed in Chapter XVIII., as it would tend to confuse the issue of the problem before us here.

FORMS OF CONTRACTION

It is not my intention to give details of the varied phenomena to be observed, especially in skeletal muscle, when stimulated in different ways or when the load is applied or removed at different stages of the course of a contraction. There are some which are necessary for our further discussion and details of the others will be found in the textbooks of Human Physiology. The articles by von Frey (1909) on striated muscle and by Grützner (1904) on smooth muscle may be consulted.

When a single electric shock is applied to the nerve of a nerve-muscle preparation, nothing happens that can be seen for a period of two- or three-thousandths of a second, as we have already learned. The muscle subsequently contracts at a certain rate and relaxes again. It is not always remembered that the processes both of contraction and of relaxation are not instantaneous. The curve can be traced, on moving smoked paper, by a lever to which the muscle is attached. The rise is gradual and so is the return. But mere inspection of the curve does not tell us whether the rate of fall was that of a body falling freely, or whether it was, so to speak, allowed to fall gradually by a gradual disappearance of the state of contraction. Tracings in which the lever is released at the top of contraction show that the rate of its fall in such a case is *greater* than when connected with the relaxing muscle, so that the state of contraction does not cease suddenly at the top of the curve, but disappears gradually. At the same time, as we shall see later, the active process of contractile stress, or, in other words, the development of energy, ceases at the top of the curve, so that changes of tension applied after this point, do not affect the total amount of energy developed.

When a muscle is held so that it cannot shorten, its contraction is said to be *isometric*, since its length does not alter; when it is allowed to shorten in such a way that it raises a weight or stretches a spring, the weight being applied in a manner such that its inertia does not come into play, or the spring such that its tension remains constant, the contraction is *isotonic*.

It will be obvious that a contraction may be of one type in a part of its course, and of another type in the remainder. Since the tension develops gradually, a muscle is able to raise a weight at a later period of its contraction, which it was unable to raise at an earlier stage. Thus the contraction is first isometric, then isotonic. On the other hand, the weight raised may suddenly come against an unyielding obstacle; the contraction is then first isotonic, then isometric. The contraction of the heart muscle is first isometric, then, after the aortic valves have

opened, auxotonic, that is, it contracts against an increasing resistance, as the arterial pressure rises.

There are several forms of experimental twitch, "arrested," "inertia," and so on, which do not concern us here.

WORK DONE

The external work done is clearly the weight raised multiplied by the height to which it is raised. Although no external work is done in maintaining the weight at this height, it is a familiar fact that fatigue results and the metabolism and heat of the muscle show a considerable consumption of energy. This point will be returned to in a later page.

When we remember that, with zero load and maximum height of twitch, no external work is done, nor when the load is so great that the muscle cannot shorten, a little consideration will show that there must be a load of a certain magnitude with which the maximal work is done; this is found experimentally to be the case.

When the muscle is allowed to relax again with the weight still on, this weight falls to its original position, so that no permanent work is done. To enable a muscle to perform actual external work, Fick devised the "Arbeitsammler" or "*work collector*," in which, by a system of catches, the weight is taken off the muscle at the height of contraction, so that the weight does not fall again; but when the muscle makes a further contraction, it catches the rim of the wheel on whose axis the weight is suspended, and raises it by a further amount and so on (see Fick's book, 1882, pp. 139-143).

Blix (1891, p. 306) has described a muscle *indicator*, which draws a curve whose ordinates are lengths of the muscle and whose abscissæ are corresponding tensions. The area of the curve is thus the work done. Similarly, in the indicator of the steam engine, the co-ordinates of the curve are pressures and volumes in the cylinder.

In order to measure the work done by an animal or man for the purpose of metabolism experiments, some form of bicycle mechanism or treadmill is generally used. A brake is applied so that the amount of work can be varied and determined. The brake may be frictional, as in the simple but accurate pattern of C. J. Martin (1914), or it may be in the form of a dynamo, as in the earlier apparatus of Atwater and Benedict (see Atwater, 1904), or again as Foucault currents, produced in a copper disc rotated between the poles of an electro-magnet, whose magnetising current can be varied (see Krogh's article, 1913).

TETANUS

When a second stimulus is applied to a skeletal muscle before the muscle has returned to its original length, the contraction due to this second stimulus starts from the level at which the first contraction is at the time, and so on for subsequent stimuli. Thus a summation is produced, by which the extent of contraction is much greater than can be brought about by a single stimulus, however strong. But each stimulus produces less increase than its predecessor, so that, after a certain number have been applied, no further increase in height results; a constant level only is maintained. This is known as "tetanic contraction."

An effect of the same kind is produced by reflex or *voluntary contraction* of skeletal muscle. A series of disturbances at the rate of 50 per second, in the median nerve of man, is sent out from the centre (Piper, 1912, p. 98). This value was obtained by leading off the muscles of the forearm to a string galvanometer. It is practically the same in the case of all the muscles tested, namely, 40 per second in the quadriceps femoris, 60 in the masseter. The shortest voluntary movements always consist of at least three or four waves. It is interesting that the frequency of these waves in the tortoise is a linear function of *temperature*, and, indeed, through the wide range from 4° to 40°. Put in other words, we may say that it is directly proportional to the absolute temperature, just as the simplest physical phenomena, such as the volume of a gas, the osmotic pressure of solutions

or the electro-motive force of a concentration battery. Yet it would seem absurd to draw the conclusion that the rate of discharge of a nerve cell has no connection with chemical processes. Similar facts in the case of the rate of the mammalian heart beat and other processes have been already referred to (page 43). A further interesting fact found by Piper in the experiments quoted is that, at 37°, the nerve cells of the tortoise have the same oscillation period as those of the warm-blooded vertebrate, namely, 47 to 58 per second.

THE NATURE OF THE CONTRACTILE PROCESS

It will perhaps facilitate comprehension of the relationship between the various experimental facts, if we first of all consider a condensed statement of the view of the processes taking place in active muscle, which the work of Hermann and others in the past, but chiefly that of Fletcher, Hopkins, and A. V. Hill in recent years, has made it necessary to adopt.

When a muscle contracts, tension is developed and external work is done if the tension is made use of to raise a weight or perform other functions requiring expenditure of energy. It is obvious, therefore, that there must be something in resting muscle which possesses potential energy of some kind, and that, on excitation, some change takes place in this system resulting in loss of potential energy. We know that lactic acid is formed and that the actual contractile process is not associated with the giving off of carbon dioxide nor with the consumption of oxygen. It is not, in fact, an oxidation, so that the "biogen" conception fails here. Although there must be some large molecules, or aggregates, containing the lactic acid group, these cannot be of a protein nature with "intramolecular" oxygen as one side chain and an oxidisable group at another place. It appears that the potential energy must be in the form of surface energy or osmotic energy, or both; at all events, in some form which is not associated with chemical reaction in the strict sense. At the end of the contraction, the cell machinery possesses less potential energy and the systems actually participating in the change, "inogens," if we may use Hermann's name, though not exactly in his sense, have let loose lactic acid.

Now to restore the system to its original state, with increase of energy content, a further, exothermic, reaction is necessary. In this process, the system is restored to its original state of high potential energy, so that the reaction by which it is effected must be one in which a considerable amount of energy is set free. This is shown by the large consumption of oxygen and liberation of carbon dioxide, indicating oxidation of some combustible substance. We have seen already (page 271) that no nitrogen metabolism is associated with muscular work as such; the oxidised substance must therefore be carbohydrate or fat. It appears that carbohydrate is normally used, but fat appears also to be capable of serving the purpose, perhaps indirectly.

After this condensed and somewhat dogmatic exposition, we may proceed to consider the evidence on which the various statements are made.

The production of *tension*, without shortening, is measured by the various methods of tracing isometric curves, as mentioned above. The principle on which these methods rest is that of arranging the muscle so that it shall pull against a strong spring or twist a stiff wire; thus the very slightest change in its own length is sufficient to produce considerable tension in the spring. This very slight movement is magnified by a long lever, or better by a reflected beam of light, whose movement is recorded on the surface of a moving photographic plate ("*optical lever*").

It is unnecessary to describe any particular experiment to show that *work* can be done by a muscle allowed to shorten. Everyday experience in the raising of weights is sufficient to prove this point.

That this work is done at the expense of *potential energy* stored in the muscle, and not by an exothermic chemical reaction involving the burning up of some food-stuff at the moment, is shown by the fact that an excised frog's muscle in nitrogen is capable of giving a maximal contraction every five minutes for two

hours and a half, before signs of fatigue appear (Fletcher, 1902, p. 491). The contractile power continues longer in oxygen, as we shall see presently. We may note here that the onset of fatigue shows that something has been used up, and that it has not been replaced. Supply of oxygen to a muscle, fatigued in nitrogen, even in the case of an excised muscle, to whose interior the access of oxygen is difficult, brings about recovery to a very considerable extent (see Fig. 135, which shows also that spontaneous rigor is hindered by the presence of oxygen).

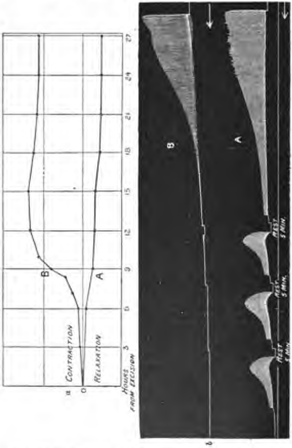

FIG. 135. LACTIC ACID DISAPPEARANCE FROM MUSCLE IN OXYGEN.

a, Development of rigor in absence of oxygen (curve *B*) and its failure to appear in the presence of oxygen (curve *A*). Gastrocnemius muscles after fatigue. Temperature, 23° C.

b, Recovery from fatigue in oxygen (curve *A*). No recovery in nitrogen (curve *B*). Sartorius muscles. Maximal break shocks, one per second. Load, 6 g. Temperature, 19° C.

(Fletcher, 1902, Figs. 2 and 13.)

In order to discover what chemical change occurs in the act of contraction itself, we must exclude the influence of oxygen. We know that contraction can take place in its absence, so that the contractile process itself does not make use of it. An important part of the problem to be solved is, in fact, the part played by oxygen. That muscles stimulated in the absence of blood supply become acid, and that lactic acid is produced by muscle as it dies and enters into rigor mortis, are old observations, but the *production of lactic acid* in the normal contraction was first elucidated by the work of Fletcher and Hopkins (1907). Surviving excised

muscle forms lactic acid, slowly, as it dies, but the fact that chiefly concerns us here is that this production is greatly accelerated by stimulating the muscle to activity. These investigators have shown that muscle freshly removed, immersed in ice-cold alcohol and disintegrated therein, contains only the trace of lactic acid which might be expected to be formed during the slight unavoidable delay in the experimental procedures. An instructive experiment, showing its production on stimulation, is described on pp. 308 to 309 of the paper referred to. Hopkins' delicate thiophene test for lactic acid is used.

This, then, is the only chemical change that can be demonstrated to occur in the act of contraction itself. It is true that carbon dioxide is slowly given off by excised muscle in an atmosphere of nitrogen and, as we saw above (page 272), Hermann represents the products of the breakdown of his inogen substance as "myosin" (that is, the nitrogen-containing residue, after separation of lactic acid), lactic acid itself and carbon dioxide. But Fletcher (1902 and 1913, p. 374) has conclusively shown that the slow evolution of carbon dioxide in an atmosphere of nitrogen is to be accounted for by the action of the lactic acid on bicarbonates already present in the muscle; the carbon dioxide so formed gradually escapes. And, what is more to the point for our purpose is that stimulation does not increase this output. Consumption of oxygen in the contractile process is excluded by the continuous and prolonged activity of muscle in its absence.

As to the *form of energy present* in muscle itself, the chief experimental evidence is contained in the work of A. V. Hill (1911, 1, 1912, 2, 1913, 1 and 4, 1914, 1 and 2) on the formation of heat in muscular contraction. There is found to be a definite proportion between the tension developed and the heat given off. If a muscle is allowed to contract isometrically at one time and allowed to shorten at another time, the heat formed is greater in the first case. It should be remembered that the heat measured in these cases represents the total energy change in the contractile process, the tension being allowed to disappear in the form of heat, and the weight raised, if such is done, is allowed to fall again. If we allow the muscle to shorten, by releasing it at the time that the maximal tension has developed, but not earlier, the heat is unaffected. The heat produced, or energy developed, is, in fact, directly proportional to the *length* of the fibres during the time that the contraction takes place and not to their volume. In other words, it is a surface phenomenon. This view was first clearly put forward by Blix (1902, p. 113). The tension developed thus depends upon the area of

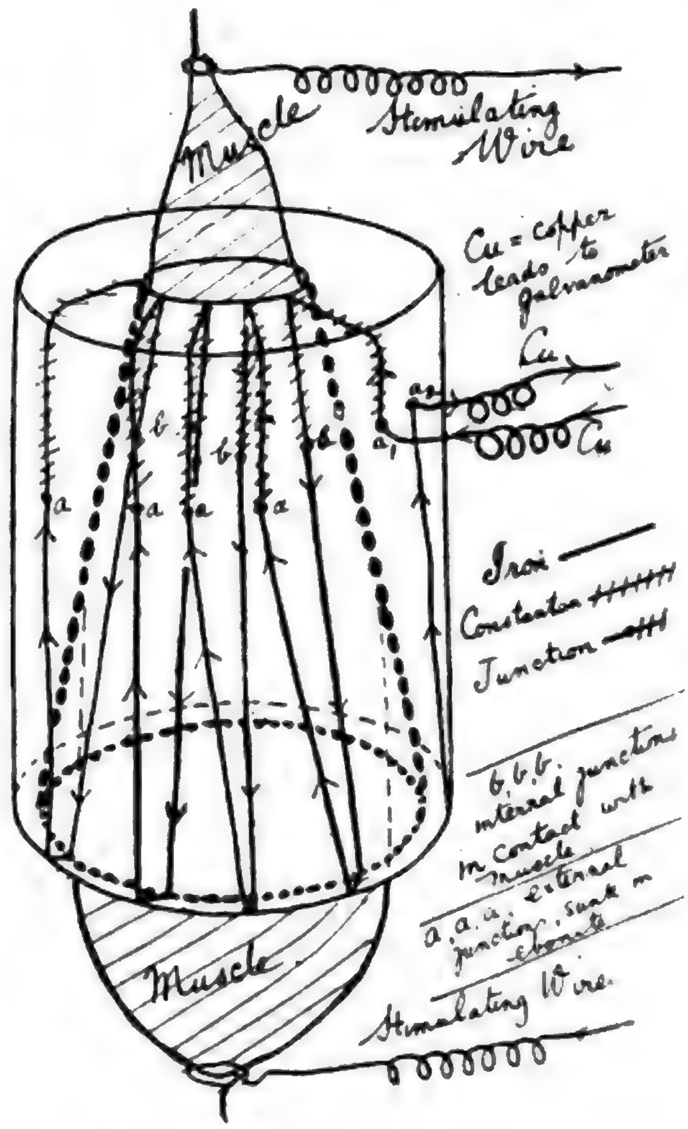

FIG. 136. SKETCH OF ONE OF THE FORMS OF THERMOPILE USED FOR THE INVESTIGATION OF HEAT PRODUCTION IN THE GASTROCNEMIUS MUSCLE OF THE FROG.

The muscle is inserted in the conical cavity, with its tendon upwards, until it fits exactly and is in contact with the junctions, *b*, *b*, *b*. When contracting, it cannot slip away from the junctions.

a, *a*, *a*, External junctions, imbedded in the ebonite.

Cu, *Cu*, Copper leads to the galvanometer.

The muscle is tied to supports at both ends, or to a lever at the lower end.

(A. V. Hill, 1913, 4, p. 307.)

certain surfaces running longitudinally in the muscle. After the maximal tension has been developed, its potential energy can be used for doing external work or may be converted into the equivalent quantity of heat. The initial process of contraction, with which we are at the moment concerned, consists in the development of this potential energy of tension, which can do work or be converted into heat. Hill has shown that, under optimal conditions, the total amount of heat developed in the actual contractile process is practically identical with that which would be derived from the energy of the tension, so that the "efficiency," in the mechanical sense, of this first process is 100 per cent. (1913, 2, p. 463).

This high efficiency is clearly sufficient to exclude the possibility of the muscular machine being a heat engine; the chemical energy of the food taken by an organism is converted into work by a more efficiènt mechanism. As we shall see presently, however, the efficiency of the total muscular process, although high, is only about half that of the first, contractile stage.

This work on the production of heat obviously requires a very perfect experimental technique, details of which will be found in the papers by A. V. Hill referred to, especially that of 1913, No. 4. A sketch of the thermopile used, in its latest form, is given in Fig. 136.

The essential process in muscular contraction is, then, the development of a certain degree of tension. As this, if unused, is converted into the equivalent amount of heat, we can, by determining the total amount of tension developed in a series of contractions, deduce the amount of heat. The application of this fact will be seen presently.

The stage in which we have now left the muscle is with a diminished store of potential energy in the complex physico-chemical system and with a certain amount of lactic acid, which has been separated from this system. In order to restore the muscle to its previous state, work must clearly be expended on it, otherwise we should be obtaining work from nothing. What do we know about the way this restoration is brought about?

We have seen that, in order to detect the production of lactic acid, the muscle must not have oxygen at its disposal, and it was definitely shown experimentally by Fletcher and Hopkins (1907) that the lactic acid disappears under the action of oxygen. It is natural to suppose that it might be oxidised to carbon dioxide and water, since, although muscular activity, in the absence of oxygen, is not associated with evolution of carbon dioxide, this gas is given off when oxygen is present.

Fletcher and Hopkins, on the ground of experiments of which Fig. 137 explains the results, thought that this was not the case, but that the lactic acid was replaced in its original position, while another substance was burnt up to afford the energy required. Subsequent work, especially by Parnas (see Fletcher and Hopkins, 1917, p. 462), showed that lactic acid is itself oxidised. The explanation of the earlier results appears to be that the production of lactic acid is a self-controlled reaction, and inhibited by acid reaction. Thus the maximum production in heat rigor only represents the normal cessation of a reaction at a critical H· ion concentration, and not the total exhaustion of the material from which it arises (Fletcher and Hopkins, 1917, p. 461). The amount of energy yielded by the oxidation of the lactic acid appears to be sufficient to satisfy requirements. It is to be remembered that the change of carbohydrate to lactic acid involves practically no loss of energy, so that the amount of energy to be obtained from the latter is as great as from the former. A. V. Hill (1914, 2) has calculated the actual amount of energy set free by muscle contracting in absence of oxygen, that is, the minimum amount which must be supplied by the restorative reaction.

In case any doubt may arise in the mind of the reader as to the origin of the lactic acid in contraction and in rigor being the same, the work of Peters (1913) may be consulted.

We must now consider the facts known with regard to the consumption of oxygen, and the evolution of carbon dioxide in the second phase of the complete muscle cycle. What do we know of the restorative phase?

In the absence of oxygen, as already pointed out, there is neither evolution of carbon dioxide nor consumption of oxygen, whereas both processes occur when oxygen is present. Further, the difference between the two cases is that, in the first case, the lactic acid remains, while in the second, it disappears. This fact indicates that the oxidation process is a stage secondary to that of

contraction and concerned with the restoration of the system to its initial state, with the disappearance of the lactic acid. The oxidation process, then, has only an indirect relationship to the actual contractile process, although it is, of course, an essential one, since it provides energy for the subsequent work to be done by the muscle in contracting. Verzár (1912) showed that the consumption of oxygen by the gastrocnemius muscle of the cat was increased for some minutes after the end of a tetanus of about half a minute; Hill (1911, 1) showed that production of heat in the frog's muscle continued for a considerable time after the end of contraction and also (1913, 1, p. 43) that, during this after-period, the heat production associated with the recovery process is about equal to that obtained in the contractile process when the tension is allowed to become degraded to heat. Peters (1913, p. 264) showed that the heat evolved when a muscle is stimulated to fatigue in absence of oxygen is about 0·9 calorie per gram of muscle. Therefore, we may reckon, from Hill's result, that the heat

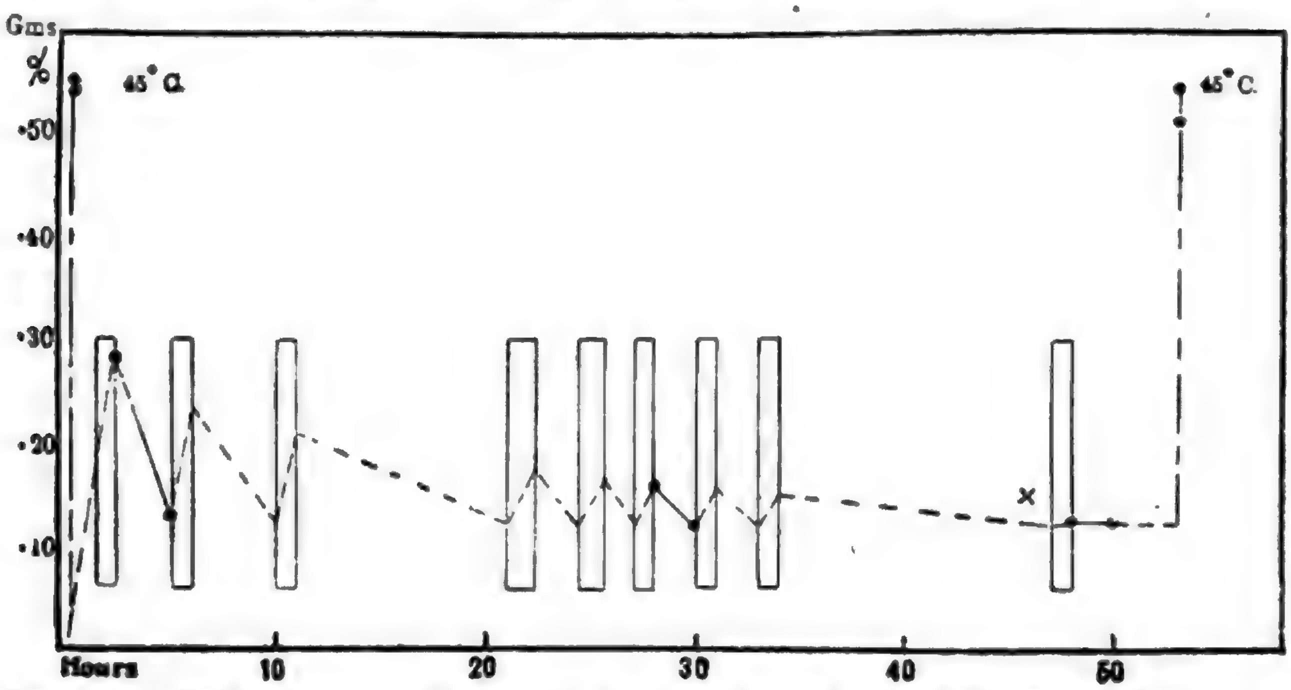

FIG. 137. DISAPPEARANCE OF LACTIC ACID IN MUSCLE AFTER CONTRACTION.—At the beginning there are two estimations of maximum lactic acid, produced by heat rigor, in control muscles. At the end there are two similar estimations which have the same value, although the muscles had gone through nine periods of severe stimulation, alternating with rest in oxygen.

The enclosed areas represent time periods of stimulation.
×, Loss of excitability.
Temperature, 15°.

The continuous part of the line shows the course of acid loss in oxygen as actually determined by estimation. The dotted line shows the presumed course of acid loss and gain during the other periods of rest and stimulation respectively.

(Fletcher and Hopkins, 1907, p. 293.)

evolved in the oxidative process of recovery is another 0·9 calorie, being equal to that produced in the contractile process. We require further to know the amount of lactic acid which disappears in the recovery process, which we can obtain from the work of Fletcher and Hopkins (1907). The maximum yield in heat rigor is 0·003 to 0·004 g. per gram of muscle and, when stimulated to fatigue about half of this (p. 280). We have now the fact that the disappearance of 1 g. of lactic acid, in the recovery process, is associated with the production of 450 calories (see the paper by A. V. Hill, 1914, 2). But 1 g. of lactic acid on oxidation gives 3,700 calories, or eight times as much as that actually obtained in the muscle process, so that, if lactic acid were oxidised in the recovery process, sufficient energy would be obtained.

The energy required to restore the high potential energy of the resting, unfatigued muscle must come from some independent reaction, involving the oxidation of a non-nitrogenous carbon compound since there are no nitrogenous products of muscular activity. But, if we accept Ostwald's position (see page 30), a "coupled reaction," in order to give chemical energy to another reaction, must have components in common with it, and it is difficult to see what these can be in

the case of muscle, if we look upon the potential energy of the "inogen" as chemical energy. Moreover, this system is not analogous to a combustible substance containing an excess of oxygen, such as nitro-cellulose, for example. There is direct evidence, as we shall see later, that oxygen is not taken up in this "intra-molecular" form, and, even if it were, the products of contraction in anaerobic conditions would contain considerable amounts of carbon dioxide, which is not the case. The formation of lactic acid from glucose or similar substance is only associated with the giving off of minimal amounts of energy. Again, as Hill points out (1913, 1, p. 77), if the lactic acid precursor could be restored in the presence of oxygen without the evolution of heat, we should be justified in concluding that the oxygen is built up into the precursor, and that the breakdown of the precursor is, in fact, an oxidation with the liberation of heat. But this is not so, "at least as much heat is used in the restoration of the contractile tissues to their previous condition as in their breakdown, so that the oxygen cannot be merely built up as intra-molecular oxygen, but must be utilised in some way in oxidation processes." In fact, one cannot imagine a chemical compound of high potential energy which would satisfy the conditions required. It would be rash to deny its existence, nevertheless, since there are such substances as nitrogen iodide. On the whole, we are driven back to the assumption of a system whose energy is more of the nature of surface energy, a view confirmed by the relation of heat and lactic acid to length of fibres. And, if this be so, the difficulty with regard to the coupled reaction vanishes.

According to the work of Parnas and Wagner (1914), there is no disappearance of carbohydrate in the second, recovery, process, whereas it does disappear in the course of contractions taking place in absence of oxygen. It appears, therefore, that lactic acid is formed from carbohydrate during the contractile phase, and oxidised in the recovery phase, but that the substance from which the acid is directly split off in contraction is not actually carbohydrate but some system of high potential energy containing lactic acid as a constituent. Another fact shown by these observers is that there is no nitrogenous substance formed in contraction, thus confirming the results of experiments on the whole organism.

We may note that, although it is carbohydrate that disappears, it passes through lactic acid before it is oxidised.

With the data at our disposal, we can obtain some further idea of the nature of this secondary oxidation process. A. V. Hill (1914, 2) has determined the total energy that can be afforded by isolated muscles stimulated in oxygen. He had previously shown (1913, 2, p. 462) how the heat production in relation to the tension developed can be estimated, and found it to be 5×10^{-6} calories (5 micro-calories) per gram-weight of tension developed per centimetre of muscle length. This is in the absence of oxygen, and refers, therefore, to the heat set free from the actual contractile process only. If the heat of the recovery process in oxygen is added, the value becomes 10 micro-calories. If we obtain a record of the tension produced in a muscle in a series of isometric twitches, we can estimate, therefore, the heat produced. Hill has compared the total heat produced when sartorius muscles were stimulated to exhaustion in air, on the one hand (that is, in insufficient oxygen), and in oxygenated Ringer solution, on the other hand. In this latter, they can be stimulated five times a minute for nearly two days, giving, on an average, 30 calories of heat per gram, whereas, as we saw, Peters found only 0·9 calorie in absence of oxygen. In Hill's experiments in air, or nearly complete absence of oxygen, the value 1·4 was obtained. Now, to afford these 30 calories, 0·008 g. of lactic acid would have to be burned. Nearly the same amount of carbohydrate would be required to produce the lactic acid, and, according to Parnas and Wagner (1914), 1 g. of muscle contains rather over 0·01 g. of carbohydrate. This is sufficient to cover the energy given off in the muscle process. We shall find presently more evidence as to the nature of the substance burned, but it would be interesting to have actual values of the carbon dioxide produced and oxygen consumed under the conditions of Hill's experiments.

Comparing again the muscle system to a gas engine, it is as if the energy of the combustion of the fuel were not used at once to drive machinery, such as drills, hammers, and so on, by means of shafting and belts, but as if an air compressor

were driven and a store of air at high pressure obtained. The engine might then be stopped and the compressed air used to drive pneumatic tools. This would be similar to the anaerobic muscular contraction. Or the compressor might be kept continuously at work, as is usual, in order to replace the potential energy of the air consumed by the tools; this corresponds to the work of a muscle under normal oxygen supply. If it be preferred to regard the potential energy of the muscle as chemical, then one might take the case of an engine driving a dynamo, which is itself charging accumulators; the current from the accumulators is then used for electric motors. In either case, the energy of the fuel is not used directly, just as that of the oxidation of lactic acid in the recovery process of muscle is not so used. The latter process is more efficient than the ordinary heat engine, since the transformation of the chemical energy does not pass through the stage of heat, although part of it appears to be lost in this way, even in muscle.

It may be mentioned here that von Frey (1909, p. 497) clearly expresses the view that only a part of the energy set free in active muscle is connected with the contractile process itself, the other part being due to a subsidiary reaction, for which oxygen is necessary.

With regard to theories of the nature of muscular contraction, we have already seen that it has some form of surface energy as an essential component of the series of changes. While, however, one set of theories regards the increase of osmotic pressure inside the fibril as the actual source of the tension, another set regards the function of the lactic acid to be that of changing the surface tension of the fibril. In any case it is clear that, in order to remove the lactic acid from its position on the fibrils, expenditure of energy must be incurred. Change of form owing to differences of water of imbibition, brought about by acid, has also been suggested. The objection to this view is that the process of imbibition under acid has the usual *positive* temperature coefficient. In the present state of knowledge as to the intimate nature of the process, any suggestions must be purely speculative. But it seems probable that hydrogen ions, arising from dissociation of the acid, play an important part in the polarisation of the cell membranes, and also in the separation of inorganic salts from adsorption by colloids in the sarcomeres, as in Macdonald's theory (1909), which is similar to that already referred to in the case of nerve. In muscle, however, these electrolytes which are set free owing to aggregation of colloids, are represented as increasing the osmotic pressure of the contents, and causing shortening by attracting water from one part of the fibre to another.

It is an experimental fact that fatigued muscle has a higher osmotic pressure than resting muscle, since it swells in a solution which is isotonic for the latter, and models have been made which shorten when distended by forcing in water. Roaf (1914) has calculated that the rate of inflow of water may be sufficiently great to offer no difficulty in this theory of contraction. It must also be admitted that, although the energy of a contraction is a function of the area of certain *surfaces* in the fibre, the fact does not necessarily exclude the possibility of the intervention of volume energy due to the osmotic pressure of the electrolytes split off from these surfaces.

The method described by Roaf (1913), by which electrodes of various types are used to detect changes in the concentration of particular ions on the surface of muscle in contraction, will probably afford valuable information, when complete, as to the time relations of the muscle processes. This investigator has found an increase of hydrogen ions, a probable increase of chlorine ions, and a diminution of oxygen tension in this way. The hydrogen ions, no doubt, come from lactic acid and the chlorine ions from potassium chloride, which might either be set free from adsorption or escape owing to changes of permeability.

There is a further group of theories which attributes the development of tension in a muscle to changes of surface tension at the contact of fibrillæ with sarcoplasm. That changes in surface tension are a controlling factor in the development of the energy of muscular contraction is made practically certain by the observation of Bernstein (1908), who found that the maximal tension developed by a particular muscle, for example, was 375 g. at 0° and 205 g. at 18°. This means that the energy in question has a *negative* temperature coefficient, and of all the possible forms of energy involved in muscular processes, surface energy is the only one that has a negative coefficient. This follows from the fact that the surface tension at the interface between a liquid and its vapour becomes zero

at the critical point, and a negative coefficient is also found experimentally (Freundlich, 1909, p. 32). In the paper by Bernstein some determinations of the temperature coefficients of the surface tensions of colloidal solutions are given, and shown to be negative (see also page 61 above).

Mines (1913, 1, pp. 14-16) brings forward good reason for regarding the production of lactic acid as responsible for the changes of surface energy, and shows that, owing to there being an optimal hydrogen ion concentration for the contractile response, the first effect of the production may be an increase of this factor to the optimal value; hence the phenomenon of the "staircase." Although the lactic acid is rapidly removed, its disappearance cannot be instantaneous, and it will probably attain a finite concentration in tetanus; at this concentration it will be produced and removed at an equal rate. This concentration is, no doubt, above the optimal one, and hence the decrease in height of each succeeding twitch in the summation of tetanus. These effects on excitability and tone are supposed by Mines to be due to the diffusion of the lactic acid, first formed at the active surfaces responsible for the production of the tension of the twitch. It will be noted, however, that we have, as yet, no explanation of the manner in which the lactic acid is liberated by the stimulus, and why the process appears to be a surface phenomenon.

A further account of the question will be found in Macallum's article (1911). It seems clear that a sufficient change in tension might be obtained from surface energy, but a decision on the point is not yet possible. In all probability, the change of surface tension is the primary factor, but osmotic pressure may play a part subsequently, although it seems somewhat doubtful whether sufficient tension could be produced by this means alone, which acts rather at a disadvantage. The movement of water, on the other hand, is most readily accounted for by changes of osmotic pressure, but it may be merely incidental.

Haber and Klemensiewicz (1909, p. 390), in their work on the forces present at the boundaries of phases, express the view of the intervention of surface tension as follows: "The relation between the chemical process and the mechanical effect of muscle is to be regarded in this way: production of acid alters the electrical forces at the phase boundary; this electrical change involves one of the surface tension also, and it is this change of surface tension that brings about the mechanical deformation of the muscle."

With regard to several of the points discussed in the preceding pages, the work of Weizsäcker (1914) gives important information. By means of a method devised by A. V. Hill and himself (1914), experiments could be made on the heat evolved by muscle immersed in various solutions. The "initial heat production" is exactly the same with or without the presence of oxygen. It is also unaffected when oxidation is prevented by potassium cyanide. With respect to the action of this substance, the facts given in Chapter XX. may be referred to. This part of the muscle process is, then, not an oxidation. It has been mentioned above that the tension developed has a *negative* temperature coefficient, and the same fact is shown by Weizsäcker to hold for the initial heat production. Alcohol prevents the development of the tensile stress, while one-third or more of the initial heat production remains. There are thus two parts or stages in the contractile mechanism; namely, one part providing free energy, and another which transforms this energy into mechanical potential energy or work. Both of these are abolished, reversibly, by the use of hypotonic Ringer's solution, and at the same time. The three different components of the act of contraction can thus be acted on. (1) Cyanide acts on the oxidations. (2) Temperature or hypotonic saline on the initial liberation of energy. (3) Temperature or alcohol on the transformation of this energy into the mechanical response. The oxidative recovery process is affected by temperature in the same way as a chemical reaction. The oxygen used increases, while the heat production falls, with a rise of temperature.

The relaxation of tone in smooth muscle is another aspect of the negative temperature coefficient of surface energy.

Pauli (1912) has developed a theory on the lines of the colloidal chemistry of the compounds of proteins with acid. I find it difficult to bring this view into connection with what we

know from other lines of investigation, and must be content with referring the reader to the lecture itself.

There is one point that requires some attention. We know from the work of Ryffel (1909) and others that, in considerable muscular work in man, lactic acid appears in the urine. This must be due to the fact that the oxygen supplied in the blood is insufficient to oxidise the whole of the lactic acid formed by the vigorous contractions before a part of it is washed away by the blood current.

In excessive work, as opposed to normal vigorous work, there is evidence of a certain amount of nitrogenous breakdown of the structure itself, as we have already seen (page 272).

Food Used.—Locke and Rosenheim (1904) found that glucose, added to the perfusion fluid of a mammalian heart preparation, gradually disappeared; but, as Evans (1914, 1, p. 408) points out, this fact does not satisfactorily prove that it was consumed, since it might have been converted into glycogen or some other substance of less reducing power than glucose. The proof was afforded by Evans himself, in the paper referred to, by showing that the respiratory quotient (see p. 279) was raised by the addition of glucose. Rohde (1910) had shown that the respiratory quotient in the first period, after setting up the preparation, was dependent on the previous diet of the animal, so that it was lower after a flesh and fat diet than after one of carbohydrate. The result was confirmed by Evans, and appears to show that the heart can also utilise fat. Experiments of Palazzolo (1913) show that the fat content of frog's muscles is diminished by tetanisation to exhaustion. If the muscle actually has the power to use fat for energy purposes without previous conversion to carbohydrate, we have a further argument that the oxidation reaction may make use of various combustible materials indifferently, and therefore that the contractile system so built up is not a chemical one. Winfield (1915), however, was unable to find any disappearance of fat in the anaerobic contraction of muscle. It may be that the production of lactic acid from carbohydrate is the normal process, but that, in the absence, or deficiency, of carbohydrate, other substances may be used and that some other acid may take the place of lactic acid. The appearance of oxybutyric acid or aceto-acetic acid in diabetes, where the muscle is unable to utilise carbohydrate, may have some connection with this possibility. A further significant fact is that Hopkins and Winfield (1915) find that the pancreas, which is so intimately connected with the utilisation of carbohydrate, has a direct effect on the formation of lactic acid in muscle, although it is a restraining one.

Campbell, Douglas, and Hobson (1914) show that muscular work is associated with a rise in the respiratory quotient, which is not merely due to production of lactic acid driving off carbon dioxide, since it remains raised during the performance of work, and, on cessation of work, there is at first a further temporary rise to nearly one. It subsequently falls to below normal. This seems to show that carbohydrate is burned. The late fall to below normal might be due either to the carbohydrate store having been exhausted or to formation of new carbohydrate from other substances.

Benedict and Cathcart also (1913, p. 94) find that there is a rise in the respiratory quotient during work; thus showing that there is an increased consumption of carbohydrate. But the fact that the respiratory quotient very rarely rises to 0·98, as they point out, prevents the conclusion being drawn from these experiments that muscular work is performed exclusively at the expense of combustion of carbohydrate. Moreover, the more severe the work, the heavier is the draft upon the carbohydrate material of the body. So that, in the subsequent resting period, a lower proportion of carbohydrate is burned for the purpose of the total energy output of this period. These workers state that their average results suggest that the energy for muscular work is afforded exclusively by the oxidation of carbohydrate. They were unable to find any evidence of the conversion of fat to glycogen during muscular activity (p. 146).

Efficiency.—We have already seen that the potential energy of tension can practically be all converted to external work, a very small fraction only being degraded to heat in the process. In other words, nearly the whole of the energy is "free." In the engineer's sense, the "efficiency," that is, the proportion of the work done to the total energy change, is nearly 100 per cent. This value

is only to be obtained in optimal conditions, but its importance is obvious. If, on the contrary, we include the heat given off in the restitution phase, as must be done when we consider the muscle as a machine performing work by means of food supplied, the efficiency is only about 50 per cent., since, in the second phase, the heat given off is about equal to the work of the first phase, while no external work is done (A. V. Hill, 1913, 2, p. 465). But even this compares favourably with the most efficient heat engine yet made.

This high efficiency of striated muscle applies only to the single twitch, or the act of raising a weight as opposed to that of keeping it *supported*. While the process of raising a weight, that is, the performance of actual external work, is a very economical one, that of keeping it suspended, without performance of further external work, is much less efficient. In the frog, as A. V. Hill has shown (1913, 4, p. 322), to maintain a particular state of tension in the sartorius muscle it is necessary to liberate six or seven times as much energy per second as that required to produce it. This fact suggests that the state of tension must be maintained less wastefully in other forms of muscular structure, and perhaps in striated muscle in natural modes of stimulation, a question to be discussed in Chapter XVIII. on "Tonus." It may be that the state of shortening is kept up without tension.

This consumption of energy in processes by which no external work is performed renders the calculation of the efficiency of the whole animal as a motor a matter of considerable difficulty. The experiments of Zuntz, Benedict, and others, on the heat and respiratory exchange of men doing measured amounts of work, are beyond the scope of this book. The paper by Macdonald (1913) may be mentioned, together with that by Glazebrook and Dye (1914), in which Macdonald's results are used to obtain mathematical expressions relating to heat and work. In the case of a particular individual, the efficiency comes out as 25 per cent. The detailed researches of Benedict and Cathcart (1913) should also be consulted by those interested.

It will be clear that the calculation of the efficiency of an animal as a motor depends on how this estimation is made. Owing to the low efficiency of the maintenance of tension, it will make considerable difference whether the calculation is made by taking the difference between the heat evolved in maintaining a weight at a constant height, and that evolved in raising it from this height to a further one, and again maintaining it at this position. The tension being the same in the two maintenance positions, the heat production will be the same, and the difference will be that associated with the performance of the external work. In this way a high efficiency is arrived at. Similarly, Zuntz calculates his values on the basis of the difference between the carbon dioxide output when walking on the level and that when walking up hill. The whole question is discussed by Benedict and Cathcart (1913). The efficiency found by them, under most accurate conditions (p. 142), that is, comparing the efficiency obtained under moderate work with that of heavier work with the same apparatus, was as high as 33 per cent. In this way an accurate base line for the increased metabolism was obtained. In other words, "the increase in the effective muscular work may be as high as 33 per cent. of the increase in total heat output."

According to Macdonald (1914), the rate of heat production, Q, associated with cycling at a uniform rate, but with varied performances of mechanical work, is expressed by the formula:—

$$x + \mathrm{E}y = \mathrm{Q},$$

where x is the heat production associated with uniform rate of movement, and y the rate of performance of work.

E is found to vary inversely with $\mathrm{W}^{\frac{1}{3}}$,
x, for a particular subject, was found to be

$$43\mathrm{V}^{\frac{\mathrm{V}}{251}}.$$

where V is the rate of revolution of the bicycle per minute. This expression also found to be related to the weight of different subjects thus:—

$$\cdot 87\mathrm{WV}^{\frac{\mathrm{VW}^{\frac{1}{3}}}{1878}}.$$

It shows that there is a particular rate of performance of work at which the total efficiency is maximal; above and below this rate, the efficiency falls.

In connection with the heat developed in tetanic contraction, the fact described by A. V. Hill (1913, 4, p. 317), that the heat-production per unit of tension is independent of the frequency of stimulation between 17 and 100 per second, is of interest. It indicates that "the rise of tension is due to the presence of chemical substances, liberated in conjunction with heat, by the processes called forth by excitation. The presence of a definite amount of these substances in the neighbourhood of certain surfaces or interfaces in the muscle, calls forth the same amount of tension independent of the exact rate at which the stimuli occur. These chemical substances are removed or destroyed at a rate proportional to their concentration at any moment; and, therefore, if they are produced (and removed) at a greater rate by an increased frequency of excitation, their concentration in the muscle must be increased proportionally. This increased concentration, however, is accompanied by an increased tension, and, therefore, the tension developed remains proportional to the rate of heat-production."

FATIGUE

If a muscle is caused to work at a greater rate than the lactic acid produced can be removed by oxidation, it becomes "fatigued," that is, incapable of full activity, or even of any at all. Naturally, this result comes on more rapidly in the absence of oxygen.

From the experiments of Fletcher and Hopkins, and of Peters, referred to above, the amount of lactic acid found in a muscle, stimulated to fatigue, only amounts to about one-half of that obtained in heat rigor. The power of contraction ceases before the whole of the "excitable substance" is used up.

This fact suggests that the lactic acid has a toxic effect, or that the process is of the nature of a balanced, reversible one. Certain experiments by Lipschütz (1908) show that the spinal cord of the frog, after fatigue in absence of oxygen, can be restored to a certain extent by perfusion with Ringer's solution carefully deprived of oxygen; so that it seems probable that the same fact would be found in the case of muscle. It would be interesting to know whether more lactic acid could be formed by stimulation, if that produced were washed away as formed. Such experiments would also throw light on the question of the formation of lactic acid from carbohydrate in the muscle.

It is important to note that fatigue of voluntary contraction, as investigated by the ergograph, or similar method, is not situated in the muscle tissue itself. Artificial stimulation of the motor nerve can still cause contraction when fatigue to voluntary innervation has set in.

The effect of the *first stimuli* after a period of rest is usually less than that of the subsequent ones; thus a series of stimuli gives, first, a rise in height of contraction (Buckmaster, 1886), then a period of maximal height, and, finally, a diminution owing to fatigue. It appears that the presence of a small quantity of the products of activity is favourable.

For further particulars of the question of fatigue and its industrial importance, see F. S. Lee (1905 and 1907) and E. L. Scott (1918), which also give references to literature.

SPECIAL CONTRACTILE TISSUES

The Heart.—There are certain important characteristics of muscular structures which are particularly well shown by the heart muscle, while there are other characteristics which have, as yet, been investigated in the case of this organ only, although they have, in all probability, a general application. It may be noticed that certain of these were, at one time, supposed to be peculiar to heart muscle, although later work showed them to be also present in nerve and in voluntary muscle. In the following pages, some facts concerning the general properties of the muscle as a contractile tissue will be mentioned; its function as a pump for the maintaining of the circulation of the blood, together with the

question of the origin and regulation of the beat, will be described in Chapter XXIII.

"*All or Nothing.*"—This fact was discovered by Bowditch (1871) in the heart of the frog. On account of its interest and importance, the words used by the investigator himself may be given, in translation, thus: "An induction shock produces a contraction or fails to do so according to its strength; if it does so at all, it produces the greatest contraction that can be produced by any strength of stimulus in the condition of the muscle at the time" (p. 687).

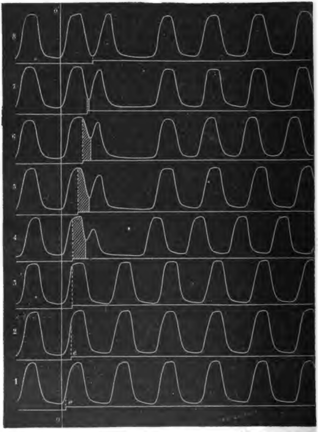

FIG. 138. REFRACTORY PERIOD IN FROG'S VENTRICLE.—Spontaneous contractions with artificial stimulus applied at various intervals after a contraction, as indicated by a rise in the signal line.

In lines 1, 2, and 3 the second stimulus has no effect.

The succeeding lines show gradual decrease of latent period (shaded part) of the extra contraction which takes place at this period.

The "compensatory pause," after the extra systole, is shown in lines 4, 5, 6, 7, and 8.

(Marey, 1885, p. 417.)

We have seen that the phenomenon is shown by voluntary muscle and by nerve. The law applies also to the movements of plants (Burdon-Sanderson, 1882, p. 42).

Staircase.—This phenomenon was also demonstrated by Bowditch (1871, p. 669) in the frog's heart. Buckmaster (1886) found it in voluntary muscle and, as we have seen (page 391), the local effect left behind by an inadequate stimulus to nerve, as shown by Adrian and Lucas (1912), seems to be a similar condition. This latter summation of inadequate stimuli ("summation of excitation," as it is sometimes called) is shown by various muscular tissues and must be distinguished from the summation of contractions observed in the superposition of tetanus, where a contraction takes its start from a position of incomplete disappearance of the one preceding it.

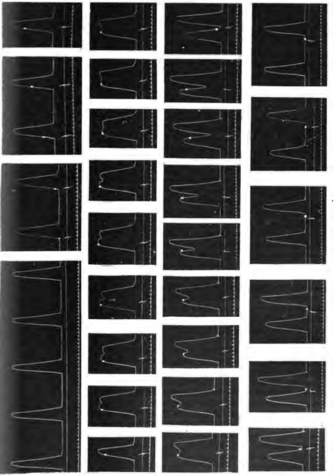

FIG. 139. REFRACTORY PERIOD AND SUMMATION IN THE HEART OF TORPEDO OCELLATA.—The exact moment of stimulation is marked by a white spot on the curve, not by the signal.

Filled with Fühner's solution. | The first five contractions are without the addition of an artificial stimulus.
Time in fifths of a second. | In the next six, the stimulus falls in the inexcitable period, so that no effect is produced.

As the stimulus comes later and later, the extra systole, at first, becomes higher and higher until it exceeds the normal one. Later, it decreases again, until, at the normal interval between two beats, the artificial contraction is only of the normal height.

The cause of the greater height of the interpolated beat appears to be that the hydrogen ion concentration is raised by the occurrence of the previous contraction to an optimal one; whereas, under the conditions of the experiment, it is below this level at the time the next natural beat occurs. The lactic acid, in fact, produced by a contraction, has usually disappeared again by the time of the next beat.

(Mines, 1913, 1, Fig. 14.)

Refractory Period.—This can easily be detected in the heart muscle. If a stimulus is put in at various points on the course of a previous contraction, natural or excited by artificial stimulus, no effect is produced until a certain stage is reached and it is found that, within limits, the stronger the stimulus, the earlier is a second contraction capable of being excited; as already mentioned, in the very earliest part of this period no contraction can be produced by any stimulus whatever. If any effect at all is produced, it is the maximum one that the tissue is capable of giving at that stage of recovery (see Figs. 138 and 139).

Summation of Contraction.—Mines (1913, 1, p. 22) shows that, in the ventricle of the Selachian fish, *Torpedo*, an artificial stimulus, at a short interval after a normal beat, produces a *greater* response than the normal one, and that this response may occur at so short an interval that the previous contraction has not completely disappeared, so that superposition may occur. As the interval increases, the height of the second contraction decreases, until, at the normal interval between spontaneous beats, the normal height of contraction is given by an artificial stimulus. These facts and the relatively short refractory period associated with the phenomenon are shown in Fig. 139 (page 453). The increase of height is, no doubt, a similar phenomenon to that observed in skeletal muscle, and is probably due to an increase of hydrogen-ion concentration to its optimal value by the lactic acid formed in contraction (see the following section below).

Action of Ions.—The powerful effect of certain inorganic ions has been referred to above (page 143). A few additional facts are of interest in the present connection. The part played by lactic acid suggests that hydrogen ions have an important share in the phenomenon. Mines (1913, 3, p. 221) finds that the optimal concentration of hydrogen ions for the heart is $10^{-7.2}$. If slightly above this, say $10^{-6.8}$, the beats become slower and weaker, the duration of the electrical change is diminished, while the rate of transmission from auricle to ventricle is decreased (see also Fig. 55, page 187).

Since increase in frequency of beat results naturally in increase of hydrogen-ion concentration, the above effects may be expected to be met with in such a case. Similarly, with increased rate of stimulation of skeletal muscle, we may expect corresponding results.

The action of calcium ions is of much importance. We have already described Ringer's work in some detail (pages 207-209). Although calcium is necessary for the occurrence of contractions, it was noticed by Locke and Rosenheim (1907) that a heart at rest, owing to absence of calcium, still continued to consume glucose, and that the electrical change still remained strong. This latter observation was confirmed by Mines (1913, 3, p. 224), and further analysed. It was found that calcium has two effects. It is well known that, as far as its effect on the contractile function is concerned, it cannot be replaced by magnesium. Thus, if we replace a normal fluid by one containing magnesium in place of calcium, the effect on the size of the contractions and on the transmission from auricle to ventricle is the same as if we had merely removed calcium; but the primary quickening of the rhythm, which is the first effect of a solution devoid of calcium, is absent. So that, as far as this latter effect is concerned, magnesium can replace calcium. It appears that the contractions fail in the absence of calcium because the actual contractile mechanism, on which lactic acid plays, is thrown out of gear in some way. A point of interest, upon which further information is required, is whether there is production of heat, which would be expected to occur when glucose is consumed. It does not seem to me to be a satisfactory explanation to suppose that the contractile substance is in the forms of strands of a calcium salt of some colloidal material, which contracts when acid is formed in contact with it. The various facts referred to on previous pages indicate rather an electrical effect on surface energy, but dogmatic statements are out of place at present.

Contractile Muscles and Arrest Muscles.—In many animals, as we shall see in more detail in Chapter XVIII., the two functions of shortening and of maintenance in the state of shortening arrived at, appear to be assigned to separate muscle fibres of different characteristic properties. In the bivalve molluscs, there is a small quickly contracting muscle which closes the shells; but the shells are kept

closed by a strong, slowly acting muscle, which follows up, as it were, the rapid contraction of the other and holds the shells together. This last effect seems to be done by some kind of an arrangement which may be compared to a ratchet, the process being attended with no consumption of energy. A description of certain of these mechanisms will be found in the book by von Uexküll (1909, pp. 92 and 144). The question is discussed in Chapter XVIII.

TRANSMISSION OF EXCITATION IN MUSCLE

In skeletal muscle it is important for sensitive grading of contraction that the fibres should act separately. In smooth muscle and in the heart, excitation can travel from one fibre or cell to another, so that there are waves of contraction passing over the mass of muscle. In the heart, the separate fibres are connected by bridges of muscular structure, but, in the typical smooth muscle, such as the intestine, it is more difficult to ascertain the mode of transmission from cell to cell. In both cases, however, it can be seen that a stimulus applied to a point starts a wave of contraction, which travels in all directions from the point stimulated.

It is very instructive to lead off the quiescent ventricle of the frog, or better of the tortoise, by two electrodes to a capillary electrometer, the electrodes being as far apart as possible, say on the apex and base, respectively. If an induction shock is applied close to one of the electrodes, say that at the base, a diphasic electrical response will be seen, indicating by its direction that a negative wave has started at the base and been propagated to the apex. The neighbourhood of the electrode at the apex is then stimulated; we see again a diphasic response, but this time the negative wave starts at the apex and is propagated to the base, so that, if the first phase in the first experiment was an upward movement of the mercury, in the second experiment it will be downward.

It is not to be taken for granted that the muscular systems of the lower invertebrates necessarily behave in the same way as the smooth muscle of the vertebrate. It is important for their movements that accurate control should be exercised over separate fibres and, accordingly, we find that (von Uexküll, 1909, p. 79), even in the Medusæ, the contraction produced by an electrical stimulus remains, in certain cases, limited to the spot excited; the excitatory process does not spread from one fibre to another. This applies to the ring of muscle around the edge of the umbrella of *Rhizostoma*. On the other hand, in *Aurelia*, as Romanes has shown (1876), the umbrella can be cut up into a spiral or other shape, and a contraction produced by stimulus applied at one end is conducted to the other end. Romanes (1885, p. 77), however, regards it as proved that the excitatory process is conveyed by the nerve network and not by transmission from muscle cell to muscle cell directly. The properties of such nerve networks will be discussed in the next chapter.

PRODUCTION OF HEAT

The time relations of the production of heat in muscular contraction have been described above. We saw that, in the restitution process, in which an oxidative reaction restores the system to its original state with a store of potential energy, a certain amount of chemical energy is degraded to heat, and also that the contractile tension developed on excitation, if unused for the performance of external work, is transformed to heat in the muscle itself.

Now, in the warm-blooded animal, this heat must not be looked upon as entirely wasted, since it serves to keep up the temperature of the organism. The importance of this raised temperature for the hastening of chemical reactions, in response to changes in the environment, has been pointed out.

Muscular contraction is, in fact, the chief, if not the only, source of heat of practical importance to the animal organism. Of course, heat is produced in other chemical reactions, especially those of oxidation, but they make up but a small part of the total.

Even cold-blooded animals and plants produce heat, but they are not provided with arrangements for keeping their temperature constant, so that it is usually only a fraction of a degree higher than that of their surroundings. Hence they are called "poikilothermic," of varying temperature, whereas the higher

vertebrates, birds and mammals, are "homoiothermic," that is, of uniform temperature.

Under certain circumstances, as in a hive of bees, the temperature of poikilothermic animals may rise considerably.

In a warm-blooded animal, even at rest, there is a considerable production of heat by muscular contraction, which is always present in the form of reflex tone. This is naturally less in sleep, and we can observe the care taken by animals to avoid loss of heat when asleep. We know also how much more rapidly we become cold when asleep than when awake. There are always certain muscular movements going on, as those of the heart and muscles of respiration.

Calorimetry.—The apparatus used for determining the output of heat is, like that used for the same purpose in physics and chemistry, called a calorimeter. It may be made on various principles, but the only satisfactory one for use with large animals, such as man, is that described by Williams (1912), as an improvement on that of Atwater, and by Macdonald (1913). The principle on which this apparatus is constructed is to absorb the heat produced by the animal by means of a current of water circulating through ribbed tubes in the chamber. When the amount of water flowing and the temperature difference between the inflow and outflow are known, the quantity of heat can be calculated. A. V. and A. M. Hill (1913 and 1914) have arranged apparatus of a similar kind for automatic registration over long periods of time and have avoided the difficulty of loss of heat, by conduction and radiation, by the use of large vacuum-jacketed flasks for small animals, and double-walled tanks for larger animals, the heat insulation in the latter case being provided by sawdust and "kapok wool." The heat produced is removed by a current of water and the difference of temperature between thermo-electric junctions in inlet and outlet is registered by a self-recording galvanometer. The micro-calorimeter of A. V. Hill (1911, 2) is used in cases of small production of heat.

The Normal Production of Heat.—A. V. and A. M. Hill (1913) find that, in adult or nearly full-grown rats, the fasting production is directly proportional to their weight. In the case of young animals, the proportion is more nearly to their surface, but is actually higher, compared with that of adults, than would be due to their relatively greater surface.

This non-proportion of heat-production to body surface in rats shows that production is not regulated by actual loss alone, but that it is a consequence of necessary tissue activity. Miss A. M. Hill (1913) points out that if the heat production is determined by heat loss alone, the former should be proportional to the difference between the animal's temperature and the external temperature. If the difference is 22° (external temperature, 15°) the mean production was found to be 203 calories per gram per day. With a difference of 11° (external temperature 26°), instead of being half the previous one, the production was found to be 166 calories, or about four-fifths.

Macdonald (1913) finds in man at rest that the heat production is proportional to the surface, that is, to the two-thirds power of the weight. In work, the effect of weight decreases, or the heat production approaches more nearly to proportionality to weight, as would be expected, since the muscles make up so large a proportion of the weight.

Effect of Food.—Fed animals show considerably more evolution of heat than fasting ones.

The Regulation of Temperature.—In warm-blooded animals, where a delicate adjustment of rates of reactions to a particular temperature has been developed, it is clearly of great importance that means should be adopted to maintain this temperature at a constant level.

Let us see what means are available for the purpose. The production may be increased or decreased by muscular activity or rest; but this, especially in high external temperatures, is somewhat limited in range, since a certain degree of muscular activity in respiration, heart beat and so on, must be continued. It is particularly effective in counteracting fall of external temperature. Next, we

have very effective means of increasing loss of heat from the surface, including that of the mouth and respiratory passages. This may be done by dilatation of the blood vessels of the skin, mucous membrane, etc., but more effectively by evaporation of water, as pointed out on page 227 above. Hence we see the value of the sweat glands. Increased evaporation of water is also caused by increased rate of breathing, which has a direct cooling action on the mucous membrane of the respiratory passages. Conversely, loss of heat may be decreased by vascular constriction in the skin.

It was pointed out by Fredericq (1882) that the cold to be struggled against comes from the outside and acts on sensory nerves in the skin; whereas increased heat almost invariably arises in the organism itself and acts by raising the temperature of the blood, in that the centres of the sweat nerves and the vaso-dilator nerves are supposed to be excited by a rise of temperature. Naturally, the effect of external cold is also to cool, in a limited degree, the blood leaving the skin, but, since one of the means adopted to counteract cold is constriction of the blood vessels in the skin, there must be comparatively little cooling of the blood. On the other hand, the external temperature very rarely rises above that of the warm-blooded animal, so that stimulation of cutaneous nerves will be secondary in this case, although not entirely excluded. Put in other words, the struggle against cold is *preventive* and obstructs loss of heat; that against heat is rather *curative* and increases the loss of excess heat produced, rarely being able to diminish production to an effective extent. As regards this last point, it may be said to depend on the condition of the animal. If the surrounding temperature is fairly warm, the animal will be quiet and thus unable to diminish muscular contraction to a further extent when the temperature rises; whereas, if the temperature is low, the animal is active and able to become quiet when the temperature rises.

Observations on the output of carbon dioxide form a very convenient means of estimating heat production and have been made much use of for this purpose. If we take an animal and measure its respiratory exchange when the temperature is at 15° in the room, the animal is active and we obtain a certain value; raise the surrounding temperature to about 30°, the animal lies quiet and probably goes to sleep; there is diminished production of heat shown by decrease of oxygen absorption and carbon dioxide output; again, lower the surrounding temperature to about 0°, great muscular activity, with shivering, sets in and considerable increase of carbon dioxide output takes place.

These experiments can be done conveniently on a mouse with the apparatus described by Haldane (1892) and modified by Pembrey (1894) for use with small animals.

Since the means adopted for regulation of temperature involve the bringing into play of so many and various kinds of efferent nerves, muscular, secretory, vasomotor, and so on, it is plain that a *co-ordinating centre* is a necessity. Experiments by Aronsohn and Sachs (1885) showed that puncture of the median side of the corpus striatum in the rabbit caused considerable rise in temperature. Further important experiments were made by Barbour (1912), who showed that application of heat or cold to the anterior end of the corpus striatum, in the region of the caudate nucleus, by means of a cylindrical metal tube through which water was circulated, caused definite changes in the body temperature. Any temperature below 33°, in the conditions of the experiments, acts as a *cold stimulus* and produces a *rise* in rectal temperature, together with shivering and vaso-constriction in the skin. Cold acts, then, as an exciting agent, like puncture, electrical stimulation, or the toxic substances of fever. *Heat*, on the other hand, that is, a stimulation temperature of 42°, in the conditions of the experiments, produces a *fall* in rectal temperature, muscular relaxation and dilatation of skin vessels (Fig. 140). It is to be presumed, also, that nerve impulses from the endings in the skin, sensitive to cold, are also in relation with the centre. No doubt, under normal conditions, the centre would be still more sensitive than in Barbour's experiments and would react to much smaller temperature changes in the blood. Barbour and Prince (1914) have shown that local heating of the centre causes diminution in the evolution of carbon dioxide, in the intake of oxygen and in the respiratory volume. Cooling the centre has opposite effects. The *production*

of heat is thus shown to be acted upon, as well as the *loss* of heat. Barbour and Wing (1913) have found that certain drugs known to produce fall or rise of body temperature, such as antipyrin, or β-tetra-hydro-naphthylamine, respectively, act, when applied to the centre itself, in much smaller amount than when given intravenously. Antipyrin causes fall of temperature with increased respiration and, occasionally, vascular dilation in the ear of the rabbit; quinine behaves similarly, with more marked vascular dilation. β-tetra-hydro-naphthylamine, on the other hand, causes rise of temperature, some shivering,

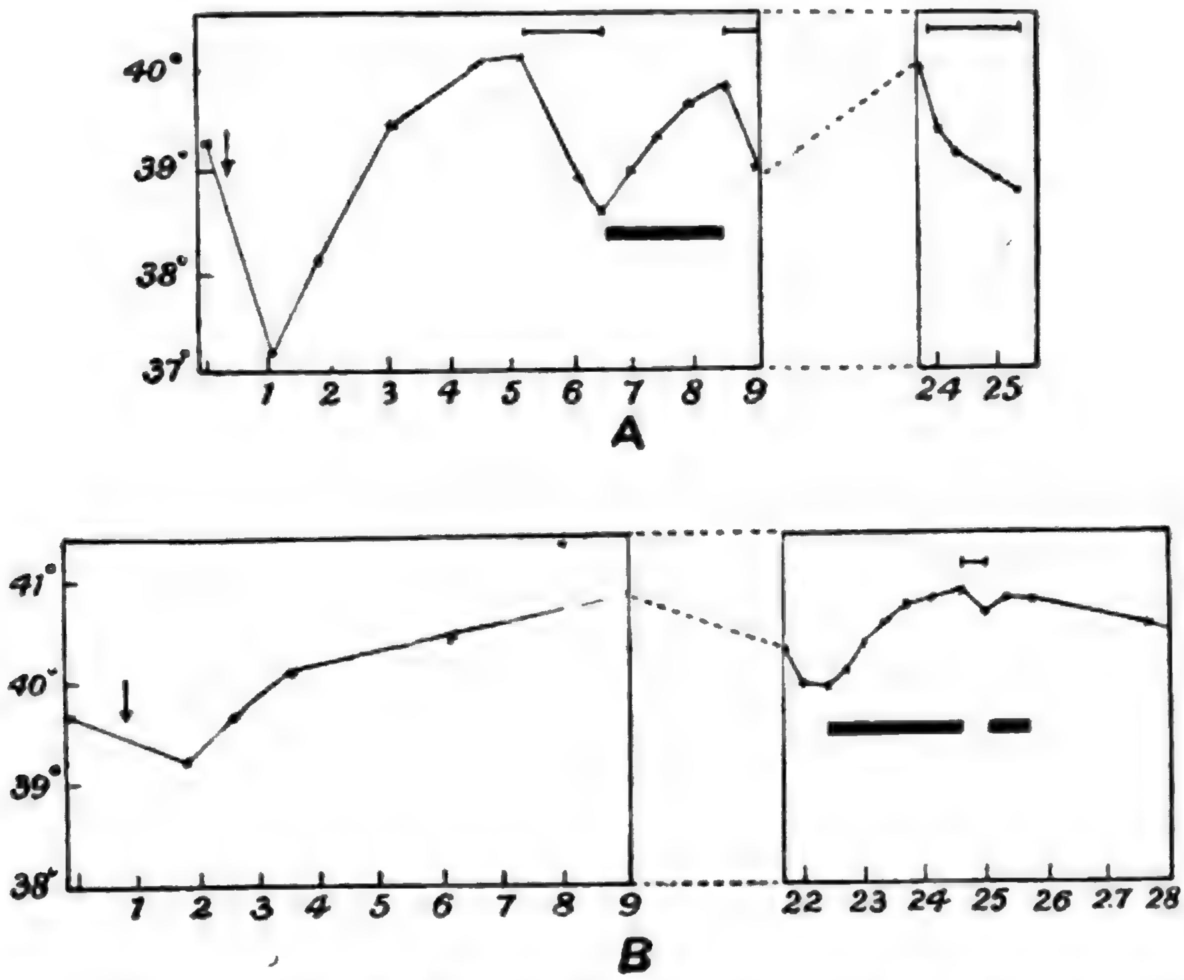

FIG. 140. CHANGES OF BODY TEMPERATURE PRODUCED BY WARMING AND COOLING THE HEAT CENTRE IN THE REGION OF THE CORPUS STRIATUM OF THE RABBIT.

Ordinates—body temperature in degrees centigrade.
Abscissæ—time in hours.

A. From one hour after insertion, at the arrow, of the tube apparatus for warming and cooling the centre to five hours. Rise of temperature produced by the injury.

During the period marked by the thin line above the curve—*warm* water passed through tube. The temperature of the body *falls*.

During the period marked by the thick line below curve—*cold* water causes *rise* of temperature.

A second passage of warm water produces a *fall*.

During the night (dotted line) the fever due to the puncture of the centre returned, but was reduced by warming the centre again.

B. The fever produced by the puncture gradually subsides and had disappeared next day. It was brought back by cooling the centre (thick line). An intermediate short period (thin line above) shows a rapid effect of warming.

(After Barbour.)

great vascular constriction in the skin and restlessness, the effects on the heat centre being probably mixed with those on other centres.

Evolution of Temperature Regulation.—According to the experiments of Vernon (1897), the carbon dioxide output of cold-blooded animals does not increase and decrease uniformly with increase and decrease of external temperature. There appears to be a region of temperature in which the output is nearly constant. Thus, on warming newts or earthworms from 10° to 22°·5, there is no increase in carbon dioxide output. This indicates some kind of control over heat production, probably in muscle, on the part of nerve centres more or less sensitive to change of temperature. A further stage of regulation is shown by the

Monotremes, as investigated by C. J. Martin (1901). *Echidna* is the lowest member of the scale of warm-blooded animals. If the external temperature changes from 5° to 35°, its temperature rises by 10°. In cold weather, it hibernates and its temperature is only half a degree above that of its surroundings. What regulation it possesses appears to be by change of production of heat. It possesses no sweat glands and exhibits no power of varying loss of heat by cutaneous vasomotor effects, nor does it increase its respiratory movements at high temperatures. The normal temperature of both *Echidna* and of *Ornithorhynchus* is 29°·8. In the latter, the temperature is maintained fairly constant, although low. It can modify both heat loss and heat production, but does not increase its respirations at high temperatures. Marsupials show a transition to higher mammals. Variation in production of heat is the ancestral method of adjustment; by this means an animal combats fall of temperature. Later, a mechanism controlling loss of heat is developed, and thus rise of external temperature is compensated for, as well as the heat produced by the animal's own activity.

For further details, with regard to production and regulation of temperature, the article by Tigerstedt (1910) may be consulted.

RHYTHMIC CONTRACTION

Many organs consisting of smooth muscle, and some with cross-striated muscle, such as the heart, exhibit, even when isolated from the influence of nerve centres, a continued series of periodic contractions and relaxations. From the facts detailed in the preceding pages, it is easy to see how a continuous stimulation might give rise to rhythmic contractions, owing to the refractory period.

Thus the ventricle of the frog's heart can be excited to rhythmic contraction by a constant current from a battery or by increase of intraventricular pressure. It may be supposed that the first application of the stimulus sets off a beat, but, for a time, the muscle is then inexcitable and, although the stimulus continues, it is ineffective. After the refractory period is past, the stimulus again becomes effective and excites a new beat and so on. It seems that the return of excitability has the same effect as a first closure of the current. Apparently, then, a constant stimulus is capable of accounting for rhythmic beats.

Another possibility to be taken into account is the using up of a store of excitable material, which has to be replaced and, when accumulated to a certain degree, discharges spontaneously. But certain objections may be made to this view.

The manner in which rhythmical effects may arise by means of a nerve network may be read in the essay by von Uexküll (1904).

The production of rhythmic movements by discharges from the nervous system to skeletal muscles will be discussed in Chapter XVI.

MOVEMENTS OF PLANTS

We have seen already how changes of permeability give rise to rapid movements in plants by allowing escape of liquid from turgid cells.

The majority of the usual movements of plants in response to light, gravity, etc., although initiated by changes of permeability, are fixed in their results by different rates of growth on the opposite sides of the moving parts. It is stated that movements due to growth, such as those of tendrils, are considerably more rapid than might be supposed, being detectable in a minute or two. The first stage of many movements is an osmotic one, as remarked, due to changes of permeability and, hence, of turgor. In such a stage, if placed in strong saline solutions, which abolish the turgor on both sides, the curvature is done away with. At a later stage, the curvature is permanent and due to growth.

There is a point of resemblance between the mechanism of plant and animal movements, otherwise apparently so different, to which attention may be called. The immediate source of the energy of the movement is, in both cases, surface and osmotic energy, although, of course, the ultimate one in the green plant is the sun's radiation and, in the animal, oxidation of material derived from plant life. In the last resort, the animal's energy is also derived from the sun.

Details of the movements of plants and the interesting facts in connection therewith will be found in Pringsheim's book (1912).

THE GRAPHIC METHOD

In the methods of analysing the forms of muscular contraction, we have, for the first time in the present book, come across a systematic use of the graphic method, so that a few words on the subject may not be out of place here.

Any representation of the relation of two phenomena to one another by drawing a curve on squared paper is, of course, a "graph." But the name "graphic method" in physiology is especially used to refer to cases where the curve is drawn by the apparatus used to observe the phenomenon. The abscissæ are nearly always time, the curve being made on a moving surface.

This surface may be of glazed paper or of glass, in either case smoked by a flat flame, supplied with gas which has passed over cotton wool wet with benzene. In this way, sufficient smoke can be deposited without burning the paper, if this is moved rapidly through the flame by rotating the drum or other surface on which the paper is fixed.

Although the present book is not primarily intended as a laboratory guide, it may be useful to mention that, when the force moving the point which scratches away the smoke is very small, such as that of the frog's auricle, it will be found very important to have as little friction as possible between the paper and the point. In such cases, the form of tracing point devised by myself (1912, 1) will be found useful. Bose (1913) has worked out a delicate method for tracing the movements of such structures as those of the leaves of sensitive plants, which have very little force. The friction of the tracing point on the recording surface is practically abolished, by causing it to move periodically in a plane perpendicular to the surface, so that contact takes place only momentarily. This vibration is effected by an electro-magnetic arrangement.

The nature of the varnish used for fixing the curves is not a matter of indifference. Ten per cent. shellac in 90 per cent. alcohol, or ordinary white hard varnish, diluted with an equal volume of alcohol, serves well, but, in either case, it is better to add a few cubic centimetres of castor oil to the litre to prevent brittleness when dry. The tracing should be quite dry when the varnish is applied, by drawing the paper through it, and it should be allowed to harden in a dry atmosphere. Further information is to be found in the article by Frank (1911).

Photographic methods have many advantages. A beam of bright light is reflected from a mirror attached to the moving part of the apparatus; any inertia can thus be avoided. The beam is passed through a slit and forms a point of light on a moving sensitive surface, paper or plate, behind the slit. Sometimes the shadow of a small moving part is projected on to the slit by means of a microscope, as in the "string" galvanometer. Details of the various methods will be found in the article by Garten (1911).

An excellent form of photographic registration apparatus for paper or plates is that made by the Cambridge Scientific Instrument Company for use with the "string" galvanometer, but is available for any form of photographic method.

SUMMARY

In the animal, there are certain tissues, known as muscular, which have the function of causing movement of parts relative to one another or, if the ends are so fixed that no change of place can occur, a state of tension is developed.

There are two chief classes of contractile tissue—the cross-striated, skeletal, which is dependent on impulses from the central nervous system to set it into activity, and the smooth, or involuntary, muscle also under the control of the central nervous system, but capable of exerting an automatic, tonic contraction or a rhythmic series of contractions. The latter class of muscular tissues, although subject to reflexes, are not under voluntary control. The muscular coat of the arterioles and the heart are examples of this class.

The rate of contraction of smooth muscle is slower than that of skeletal muscle; but numerous varieties occur in both, so that the extreme cases do not greatly differ

The intimate structure of muscle fibre is very difficult to investigate. The dark bands seen in cross-striated muscle are doubly refracting. In the state of contraction, the dark and light bands appear to change places; but the position of the doubly refracting part does not change, so that it is the light band which is doubly refracting in this state. According to Engelmann, in contraction the doubly refracting part increases in volume at the expense of fluid derived from the singly refracting part. The property of double refraction is, according to Engelmann, associated with the power of contractility as a general rule in the animal kingdom.

The production of tension is the essential point in the mechanics of muscular contraction. The muscle changes its properties from those of an unstretched steel spring to those of a stretched one, without necessarily changing its length.

Various modes of contraction may be obtained from muscle, according to whether it is allowed to shorten or not, or the phase of the contraction at which the muscle is allowed to shorten, or at which the load is applied or removed.

An isolated muscle can be made to do external work by raising a weight, which is prevented from falling again. This is done by a mechanism known as the "work collector." The work done by an animal is measured by some form of "ergometer."

A stimulus applied before the effect of a previous one has disappeared produces a contraction which is itself less than the previous one, but takes its origin from a shorter state of the muscle. Since the effect of each is less than that of its predecessor, a stage is reached beyond which no further shortening takes place. If the stimuli succeed one another at a rate such that the muscle has not commenced to relax before the next stimulus arrives, we have a smooth continuous curve. The phenomenon described is known as the "summation of contractions," producing "tetanus."

This tetanus is also the condition of skeletal muscle when excited by impulses from the cells in the nerve centres. The rate at which these impulses are sent out is, in man, from forty-seven to fifty-eight per second. In the tortoise, the rate is a linear function of temperature between 4° and 40°, like that of the mammalian heart between 27° and 40°.

A resting excitable muscle is a physico-chemical system possessing potential energy. When stimulated, this potential energy is converted into energy of tension, which can then be used for the performance of work, or allowed to become degraded into heat.

This contractile process itself is associated with the splitting off of lactic acid, but there is neither consumption of oxygen nor evolution of carbon dioxide. It is not an oxidation process.

To restore the potential energy which the system has lost in contracting, energy is supplied by another reaction of a chemical nature, which succeeds the contractile stage.

The lactic acid separated in the first process is oxidised to afford energy for the second, recovery, process. There is consumption of much oxygen and evolution of carbon dioxide in this latter phase.

The energy developed in contraction, as measured by the heat into which it is converted, is directly proportional to the tension produced. It is proportional to the *length* of the fibres at the time the contractile process takes place and not to their *volume*. It is, therefore, a surface phenomenon. Osmotic energy may, nevertheless, intervene as a further step, being controlled by the products of the change in surface energy.

The fact that the reaction by which the energy of the contractile system is restored is one having, apparently, no chemical component in common with the contractile system itself, indicates that this latter is not a chemical system, but

one of a more physical nature. The nature of its energy as that of surfaces is also confirmed by the fact that it has a *negative* temperature coefficient, while all other possible forms of energy involved in muscular contraction have a positive one.

The muscular system is analogous to that of a gas engine used to compress air into a reservoir, from which it is taken to drive, by its pressure, various machines and tools. The energy of the oxidation of the fuel is not used from the engine directly.

There is reason to believe that it is the lactic acid or its hydrogen ions that is responsible for the changes in surface energy.

The food consumed in muscular contraction appears to be, normally, carbohydrate; but there is evidence that fat may be used. Nitrogenous compounds do not act as food directly.

The "efficiency" of the first, contractile, phase is practically 100 per cent., that is, the whole of the tension developed can be used for work. That of the whole process only amounts to about 50 per cent., since part of the chemical energy of the oxidation process of the restoration period is lost as heat.

The efficiency of the act of maintaining tension, as by holding up a weight, is much less. This fact renders the calculation of the efficiency of the whole animal or of an isolated organ, such as the heart, a matter of difficulty.

The heart muscle shows particularly well certain characteristics which are known to apply to all muscle, and others which probably apply. These phenomena are: "all or nothing" in respect of strength of stimulus; "staircase," by which, after a rest, successive contractions increase in height for a time, and probably due to increase of hydrogen ions to the optimum point; "refractory period," as already described for nerve; in certain conditions, summation of contraction; and great sensibility to certain ions, especially those of hydrogen.

There is no transmission from fibre to fibre in skeletal muscle. It does, however, take place in smooth muscle. It is not certain whether, in all cases, the spread of excitation in all directions is conveyed by an intramuscular nerve network, although it appears to be done by that of the Medusa.

In warm-blooded animals, heat produced in muscular contraction is utilised for the purpose of keeping up the temperature. The production of heat is measured experimentally by specially constructed calorimeters, which also allow the respiratory metabolism to be determined simultaneously.

In adult animals at rest the production of heat is proportional to the external surface, that is, to the loss. In work, since the muscles are producing heat in excess, it approximates more to proportionality to weight.

The temperature is regulated either by change of production, that is, by greater activity or rest, or by change of loss, as by cutaneous vascular changes and by evaporation of water in sweat and expired air.

The co-ordinating centre of these factors of regulation is in the corpus striatum, and is so arranged that it is sensitive to changes of temperature in the blood. When warmed, this centre responds by causing muscular relaxation and dilatation of skin vessels, thus producing a fall in body temperature. Conversely, when cooled, it causes a rise in body temperature by exciting muscles to shivering and by vascular constriction in the skin. It is probably also sensitive to afferent impulses from heat and cold receptors in the skin.

A certain degree of control of heat production appears to be the earliest form of regulation, and is present in a rudimentary form even in cold-blooded animals. As far up as Echidna, there is no other mechanism.

The possible modes of origin of rhythmical contraction are discussed in the text.

In plants, movements are produced by changes of turgor, due to changes of

permeability, on excitation by various means. These changes of form are, in many cases, afterwards fixed by differences in rate of growth.

A short account is given in the text of the graphic method, as used in physiological work, together with some practical details.

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CHAPTER XV

NERVOUS SYSTEMS, PERIPHERAL AND CENTRAL

The necessity of means of bringing into relation with each other the different parts of an organism, as it grows in size and complexity, has been already alluded to (page 378). Moreover, the same muscles may require to be put into action, say for flight, when an animal either sees an enemy or is touched by one, or for the obtaining of food. If there were nerve channels directly connecting every sense organ with every muscular group, the multiplicity of communications would be most wasteful, besides inefficient for its purpose. The comparison of the central nervous system to a telephone exchange is often made, and is quite appropriate. Any subscriber can be put into communication with any other. Similarly, in the animal, impulses arriving from a particular source may be, according to circumstances, connected up, as it were, to different muscular mechanisms. It will be seen that, since the arrangements of a telephone exchange are mainly a matter of wiring, so, in great part, the study of nerve centres consists of the anatomy of tracts of nerve fibres; a study which tells us of the possible ways of communication between the centres controlling various parts. This aspect of the subject can best be studied in textbooks devoted to it, and will only be treated incidentally in this book. An account, in some detail, will be found in Starling's book (1920, pp. 315-432).

There is another aspect, which is of a more general nature, and consists in the investigation of the means by which the functional use of the paths provided is arranged for.

The co-ordination of the activities of various parts of the organism, or integration, as the essential function of the central nervous system, is especially insisted on by Sherrington (1906). When we consider that in the organism, just as in the community, progress depends on the most effective working together of the component units for the common good, we see how, as Gaskell has shown (1908), the nervous system has been the dominant factor in evolution. Other systems have been modified and changed in function in order to give opportunity for the growth of this pre-eminently important one. As Gaskell puts it (p. 19), "The law of progress is this—The race is not to the swift, nor to the strong, but to the wise."

ORIGIN OF THE NERVOUS SYSTEM

Different views have been put forward with regard to the way in which nerve centres first made their appearance. A brief account will be found in the paper by G. H. Parker (1911). The most satisfactory hypothesis seems to be that of this investigator, who finds that sponges, although no nervous structures are to be found in them, and although they exhibit none of the characteristic rapid reactions of animals with even the most primitive nervous system, do nevertheless show contractile response to stimuli. The opening and closing of oscula takes place in response to movements of the sea water and is brought about by contractile tissue, similar in its appearance and slowness of response to smooth muscle in the higher animals (see Parker's paper, 1910).

Muscular tissue makes its appearance, then, before nervous tissue. Even if we regard the sponges as arising from protozoa by a branch which is separate from that taken by the Cœlenterates, it must be admitted that their organisation is a

more primitive one than that of the latter and nearer to the ancestral forms. Parker's theory regards the muscle cell, or "effector," as developed from amœboid epithelium and as being gradually displaced to form a layer underneath the external epithelium. "Next in sequence would appear the receptor or sense organ which, derived from the cells in the neighbourhood of a developed effector (see Fig. 141), would serve as a more efficient means (D) of calling this organ into action than direct stimulation. This stage is represented by many Cœlenterates; and their quick responses, as compared with those of sponges, are dependent, I believe, upon this advance in organisation. Finally, in forms somewhat more advanced than the Cœlenterates, central nervous organs or adjusters would begin to differentiate in the region between the receptors and effectors; and these would develop in the higher animals first, as organs of transmission whereby the whole musculature of a given form could be brought into co-ordinated action from a single point on its surface and, secondly, as the storehouse for the nervous experience of the individual and the seat of those remarkable activities that we recognise in the conscious states of the higher animals. Thus nerve and muscle did not develop independently, as claimed by Claus and Chun, or simultaneously, as maintained by Kleinenberg and the Hertwigs, but muscle appeared first as independent effectors and nerve developed secondarily in conjunction with such muscles, first as a means of quickly setting them in action and, secondly, as a seat of intelligence" (Parker, 1911, pp. 224-225). The same author further points out (1909, p. 58) that a receptor or sense organ alone would be of no service to an organism, neither would nerves nor nerve centres alone, whereas a muscle cell, or effector, is of use, if it can be stimulated directly. It is thus not improbable that there should be primitive multicellular animals possessing effectors only and neither cells sufficiently differentiated to be called receptors nor other nervous mechanisms.

The next step in evolution after that of the sponges is the receptor-effector system, as seen in its simplest form in the *sea anemones*, and, with more complication, in the jelly-fish. The outer surface of a sea anemone is found to be diversely sensitive for different kinds of stimuli and, moreover, the response to stimulation of a tentacle may be a movement in a distant part of the organism, without any movement of intermediate regions, so that something in the nature of nervous transmission is present. On histological examination, the skin of these animals is found to consist of three layers. An outer one contains epithelium cells modified to serve as sense receptors, having bristles on the outer ends. Their inner ends are prolonged into finely branched processes, clearly of nervous nature, which intermingle with those of other cells to form the second, nervous, layer. This layer also contains cells with branched processes, which intermingle with the rest, in fact, ganglion cells. It appears that this layer constitutes a true nervous network, continuous over the whole body, and that no centralisation of the adjuster mechanism has yet taken place. The third layer consists of muscle cells in contact with the nerve network. Experiments of various kinds show that the reactions of one part of the body do not serve as experience for another, that is, it shows no evidence of what we should call a central nervous system (von Uexküll, 1909, p. 73). Its neuro-muscular system consists of receptors and effectors, united by a nerve network, which is composed of the processes of receptor cells and of ganglion cells contained in the network.

The *jelly-fish*, owing to their locomotion, lead a life subject to greater variety of experiences, and we find specialised forms of receptor organs, which are much more sensitive than those of the sea anemone. The muscular band is under the control of a nerve network, which receives numerous fibres from the receptor organs. This network conveys the excitatory process from one part to another, since contraction can pass from one group of muscle fibres to another over a gap containing network but no muscle fibres. It also conveys excitation in all directions; if all the sense organs but one are removed, the rhythmic impulses to swimming movements are started by this one and radiate from it in all directions.

Proceeding upwards, we find in the earthworm a centralised nervous system, a diagram of which, taken from the description and figures of Retzius (1892), is given in Fig. 142. In this animal we have a cerebral ganglion, or brain, in the

anterior end, and a segmented nerve cord passing along the ventral middle line and extending to the posterior end. The integument contains many sense cells, each giving rise to a single nerve fibre, which enters a ganglion of the ventral nerve chain and divides therein into numerous branches, forming the so-called neuropile. Whether this neuropile is actually a network, that is, whether there is actual physical continuity between the branches of different cells, is difficult to make out. But it seems that we have here a definite system of "neurones," which play so large a part in the higher nervous systems.

The name "*neurone*" was given by Waldeyer (1891, p. 1352) to the elementary unit of which the higher nervous systems are built. A motor neurone consists of a cell body, with a nucleus, a nerve fibre conveying excitation from the cell body outwards, and which may be long or short, together with a number of branched processes or "dendrites," receiving impulses from outside the cell. The nerve fibre process usually ends in a branched form either on the cell of an effector organ of some kind, muscle, gland cell, etc., or on another neurone. In the sensory neurone, the long fibre usually receives the impression from the exterior and conveys it to other neurones by the dendrites. The peculiarity of a neurone, as compared with other cells, is the possession, in the larger animals, of great length; it may have its cell body with nucleus in the brain at the anterior end of an animal of several feet in length, while the termination of its outgoing process, the axis cylinder process or axon, may be on a nerve cell at the distant end of the spinal cord. "Neurone" should have the final e, making the o long and suggesting that it is made up of parts. The names of these parts have a short o. "Centron" is the body of the cell; "axon," the nerve fibre; "dendron," any other process; and "dendrites," the fine branches of the dendron (Bayliss, 1916, 2).

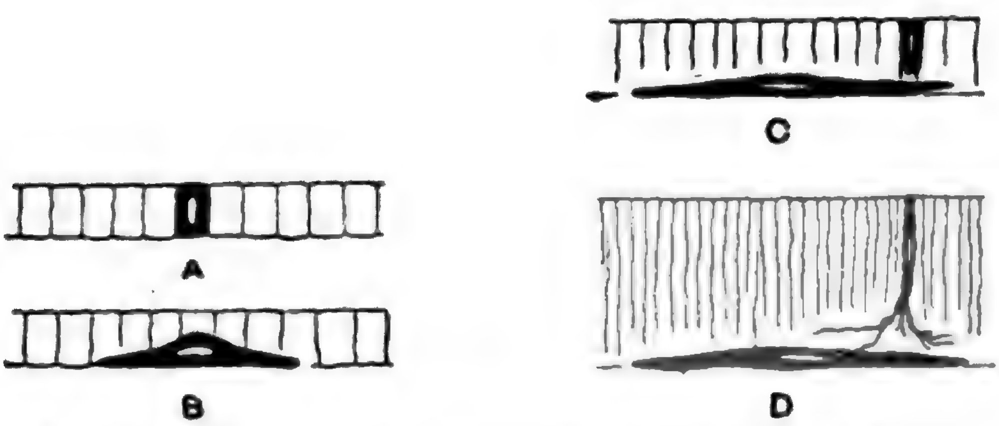

FIG. 141. DIAGRAM TO ILLUSTRATE THE EARLY STAGES IN THE DIFFERENTIATION OF THE NEURO-MUSCULAR MECHANISM.

A, Epithelial stage.
B, Differentiated muscle cell at stage of sponge.
C, Partially differentiated nerve cell in proximity to the fully differentiated muscle cell.
D, Nerve and muscle cell of Cœlenterate stage.

(See text, page 465.)

(Parker, 1911, p. 222.)

In the earthworm, then, we have a primary sensory neurone, with its cell body in the skin, and its nerve process ending in ramifications in the neuropile of the segmental ganglia. In these ganglia, we find also large nerve cells, whose thick axons pass out as motor fibres to the muscles of the body wall. These motor neurones also are possessed of dendrites, which contribute to the formation of the neuropile and form connections, either of direct continuity or merely of contact, with the endings of the sensory neurones. This is the simplest possible *reflex arc*, sensory impressions giving rise to a motor response.

In the central nervous system of the earthworm, we find also *association neurones*; these have processes serving to connect neurones within one ganglion, or from one ganglion to another, but rarely extend over more than two segments. These neurones do not pass out of the central nervous system, and it may be noted here that the complexity of this system, with rise in the scale of evolution, depends on the number and length of these association neurones; so that we reach finally the cerebral cortex of the higher apes and man, which consists entirely of this kind of neurones, having no direct connection with the exterior. It is not difficult to understand why specially sensitive and elaborate receptor organs should

be developed at that end of an animal which is most exposed to the complexity of outer influences. The cerebral ganglia have, no doubt, been formed in relation to these manifold sensory impressions, and long association neurones have been developed in order that slight influences, able to affect the delicate receptors in the head but not the less sensitive ones elsewhere, may affect distant parts of the organism.

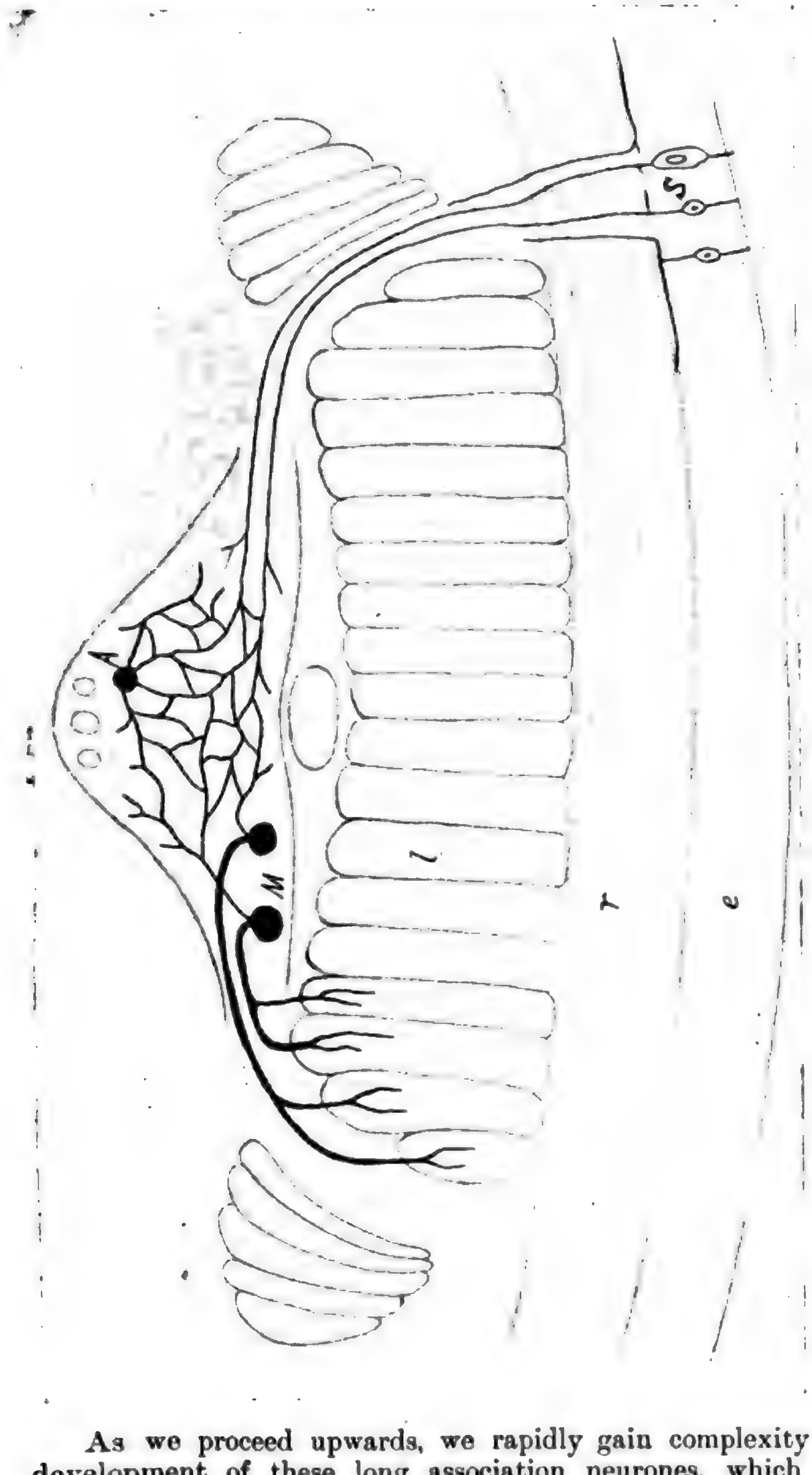

FIG. 142. DIAGRAM OF THE NERVOUS SYSTEM OF THE EARTHWORM, SIMPLIFIED FROM THE FIGURES OF RETZIUS (1892).—Cross section through ventral part of animal, including a ganglion.

l, Longitudinal muscles.
r, Circular muscles.
e, External epithelium, with sense cells.
A, Association neurone, usually with long processes at right angles to the plane of the paper, passing up and down the chain of ganglia.
M, Motor neurone.
S, Sensory neurone.
In the middle of the ganglion, the "neuropile," composed of network of processes of the three kinds of cells referred to.
To avoid confusion, each of the two lateral nerve trunks is represented as consisting of one kind of fibres only, sensory on the right, motor on the left, whereas both nerves are really mixed.

As we proceed upwards, we rapidly gain complexity and efficiency by the development of these long association neurones, which are indeed the only fundamental difference between the higher and lower invertebrates. In the vertebrates, the primary motor neurones have their cell bodies in the central mass, like the invertebrates, but the primary sensory neurones, instead of having their cell bodies in or near the surface, have undergone a change in situation, so that the cell body is placed, as it were, on a side branch of the nerve fibre close to

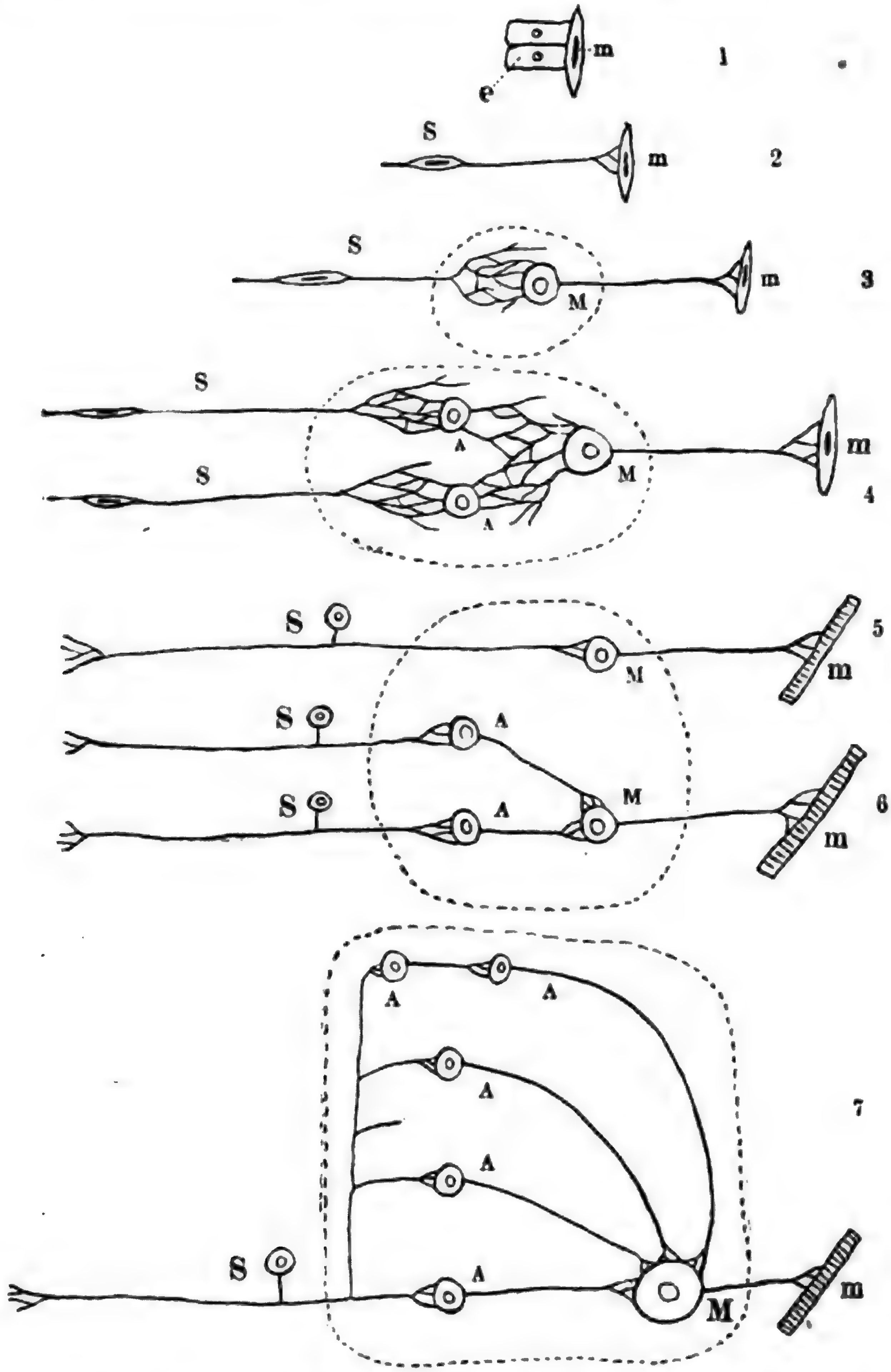

FIG. 143. DIAGRAMS OF THE EVOLUTION OF THE CENTRAL NERVOUS SYSTEM.

S, Sensory neurone. *A*, Association neurone. *M*, Motor neurone. *e*, Epithelial cell. *m*, Muscle cell. The dotted lines indicate the boundaries of the nerve centres.

1. Sponge.
2. Sea anemone.
3. Simplest form in the earthworm.
4. Intercalation of association neurones in the earthworm.
5. Exceptional, simple, reflex arc in vertebrates. Possibly existing in the case of the knee jerk.
6. Usual type in vertebrates. The cell bodies of the sensory neurones are in the dorsal root ganglia, instead of in the receptor organs, except in the olfactory organ.
7. Addition of higher centres, consisting only of association neurones, some of which are inhibitory. They form, as it were, longer and longer parallel or alternative loops between the receptor and effector organs. These loops may be followed in Fig. 147.

the central nervous system, and thus the ganglia of the dorsal roots are formed. The olfactory nerve alone retains its primitive condition.

Whether there is any actual continuity, of the nature of that presumed to exist in a nerve network, in the case of the neurones forming the nerve centres of the vertebrate, has been somewhat disputed. The evidence is of a very doubtful character, and the relation by contact with the interposition of a "*synaptic membrane*," as it is called by Sherrington (1906, pp. 15-17), becomes marked and predominant.

When the axon is cut off from its cell body, it undergoes a process of *degeneration*, as a part of any cell, except that containing the nucleus, does. Owing to the large number of long neurones in the vertebrate, section of their nerve centres results in extensive degeneration, contrary to what obtains in animals like worms. The degenerations following known, localised injuries form an important method of investigating the manner of connection of one part of the central nervous system with another.

A fact to which Parker calls attention (1909, pp. 338-345) is the origin of effectors, other than muscles, such as chromatophores, glands, phosphorescent organs, etc., which, like that of muscle itself, appears to be independent of that of the central nervous system and to be continued throughout the course of evolution. These effectors become appropriated by the nervous system, as it grows in its power of control.

Many of the facts referred to above may be made clearer by reference to the diagrams of Fig. 143.

The most striking morphological fact with regard to the vertebrate nervous system, as compared with the invertebrate, is that, although the cerebral mass or brain in both cases lies in front of and above the alimentary canal, the continuation along the body—spinal cord in the vertebrate, ganglionic chain, or similar set of ganglia, in the invertebrate—has a different relationship to the alimentary canal, being dorsal to it in the vertebrate, ventral in the invertebrate. Now Gaskell (1908) has brought forward a theory, supported by a large body of evidence and entirely in agreement with the pre-eminence of the nervous system, according to which the vertebrate nervous system is in direct continuity of descent with that of the invertebrate. The ventral ganglion masses grow up, as they increase, to surround the primitive alimentary canal, which finally becomes the central canal of the spinal cord in connection with the ventricles of the brain. This necessitates, of course, the formation of a new alimentary canal, which takes place by enclosure of a space by downgrowth of the body walls ventrally. There is really no more difficulty in this, as Gaskell points out (1908, p. 58), than in the production of a new respiratory system in the passage from fish to land amphibians and less difficulty than in supposing that a new nervous system was developed. The way in which this view accounts for many curious features in the embryological development of the vertebrate nervous system can only be appreciated by consultation of Gaskell's book.

STRUCTURE AND PROPERTIES OF THE NEURONE

We may next proceed to consider, briefly, some facts as to the general structure and function of the elementary constituents which make up the nervous system, omitting, for the present, the question of the nerve network.

One of the most striking characters of the neurones, at all events in the higher vertebrates, is that, contrary to the cells of other organs, the whole of those which the adult animal is to possess are present at birth, gradually taking on functional activity. There is no evidence of any regeneration after destruction or death of any individual neurone. Although there are great varieties in detail, especially in size and shape, in the neurones of different function, they all consist of a nucleus surrounded by cytoplasm, and have an unbranched process which may, however, divide peripherally—the axon; they have also branching processes, the dendrites, which also communicate with those of other neurones, as mentioned above.

Certain observers, by examination of nerve cells fixed and stained in various ways, have shown that, after this treatment, there are two obvious structural

elements in the cytoplasm: (1) Large granules or masses which stain deeply with "basic" dyes, and are called, from their discoverer, "Nissl Bodies," and (2) an appearance of fine fibrils passing through the cell substance from one process to another, "neuro-fibrils." Now Mott (1912) and Marinesco (1912) have made very careful examinations of living nerve cells, partly by the aid of the ultramicroscopic method of brilliant dark-ground illumination (see page 82), and agree in the statement that neither Nissl bodies nor neuro-fibrils are present in the living state; a result which might be suspected from the work of Hardy described in Chapter I. of this book (see Fig. 144). The considerations of that chapter as to cell protoplasm in general apply to that of the neurone. It is seen to be full of fine granules, behaves as a viscous fluid, and, to reagents, responds as an electro-negative colloid. Without denying the value of observations on fixed cells, Mott

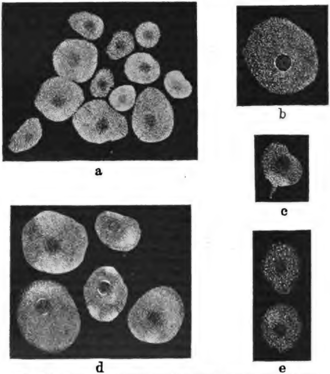

FIG. 144. LIVING NERVE CELLS FROM THE DORSAL ROOT GANGLIA, AS SEEN WITH THE ULTRA-MICROSCOPE.

a, Normal cell, from dog three days old. Fine colloidal particles in cytoplasm, showing Brownian movement. Nucleus appears nearly empty. No indication of Nissl bodies, nor of neuro-fibrils. No amœboid movement of the cell as a whole was noticed.

b, Similar cell from new-born dog. More highly magnified. Collection of particles around nucleus.

c, From ganglion in lumbar region of new-born dog. Large, brilliant granules are seen where the axone leaves.

d, Five cells from small dog. They showed spontaneous changes under observation. The brighter areas, due to larger particles, changed their positions in the cells.

e, Two cells, which had been treated with sodium iodide for eight hours. Appearance of larger aggregates of particles, surrounded by smaller ones still showing Brownian movement.

(Marinesco.)

thinks that the conceptions of neuro-fibrils and Nissl substance are apt to lead astray in the interpretation of phenomena taking place during life. His observations were made on ganglion cells mounted in serum or cerebrospinal fluid of the animal whose cells were under observation, and they were kept at body temperature. The granules are extremely minute, not more than 1 μ in diameter, and they appear themselves to consist of a colloidal solution surrounded by a lipoid envelope. This envelope stains deeply with methylene blue.

The observations of Ross Harrison on the outgrowth of nerve fibres have already been mentioned (page 22). They suggest strongly an amœboid movement of the processes of the nerve cell, at all events in the embryonic state, and show that the nerve fibres grow out from cells in the central nervous mass, thus placing the neurone doctrine beyond question (see Fig. 20). If this amœboid nature of the branches of the cells continues in the adult, the possibility is present of influence by changes of surface tension, due to excitation processes in the cell, and an effect on the degree of contact with other neurones.

Contrary to the nerve fibre itself, the cell body of the neurone is very sensitive to deprivation of oxygen, both cytoplasm and nucleus becoming swollen. Similar changes occur when the axon is injured, and the power of recovery depends on the degree of the injury. If recovery takes place, the axon grows out again to the periphery.

From the effect of raised temperature, Mott concludes that there are at least two colloidal substances in the living cell, fluid granules with delicate membranes and a viscid homogeneous semi-fluid substance forming the external phase. The membranes on the surface of the internal phase are, doubtless, produced by adsorption. Rise of temperature causes the granules to blend with the external phase.

The fact that each lateral half of the electrical organ of Malapterurus is innervated by one single large efferent neurone enabled Gotch and Burch (1896) to investigate certain elementary properties of the mode of discharge of the nerve cell. Although caution must be exercised in extending these results to all efferent neurones, they give valuable indications of the phenomena possible. The response to a peripheral stimulus is usually multiple, that is, a rhythmical series of discharges. In fatigue, the number of discharges per second is decreased before the intensity of each individual discharge falls. The time taken by an impulse to pass from the afferent to the efferent side of a cell is from 0·008 to 0·01 second and is increased by fatigue.

It is somewhat difficult to state what is actually the function of the cell body of the neurone, apart from being the meeting place of fibres from various other neurones and thus allowing for the connection of a number of afferent arcs with the same "final common path." It seems that it must act in reinforcing impulses which might be too weak to set up a disturbance in a fresh neurone. The refractory period, no doubt, plays a part and we must also suppose that changes in the cell body are able to prevent the reception of impulses coming from sources extraneous to those connected with the particular act in which the neurone is engaged at a given moment. In certain cases it seems that it is not necessary that the impulses should pass through the actual cell body itself. When this lies, as it were, on a side branch of the nerve fibre, the continuation of this fibre may branch and act as dendrites to form connection with another neurone. This fact has only been clearly shown for one case, namely, that of the crab, in which the cell bodies lie on the surface of the ganglion mass, and Bethe (1897 and 1898) has succeeded in cutting them off, leaving the reflex to be conveyed through the neuropile. After a time the reflex disappears, presumably because the trophic action of the cell body with its nucleus has gone.

A less convincing experiment of the same kind was made by Steinach (1899) on the dorsal root ganglia of the frog. By separating these ganglia from their blood supply, it was found that the cells degenerated after about fourteen days, but that the sensory impulses were still transmitted through the ganglia. If these results apply to neurones in general, it must be admitted that the actual cell substance itself has very little function, except that of nutrition, and the main physiological activities must be relegated to the synapses.

The cell bodies have the usual needs of cells undergoing metabolic changes,

especially the need for oxygen. The question of the *respiratory exchange* of the central nervous system has not been thoroughly investigated. Leonard Hill and Nabarro (1895) found the oxygen consumption and the carbon dioxide evolution to be considerably less than that of muscle, as would be expected. The rate of the blood current was not taken especially into account in these experiments, so that it is difficult to compare the absolute respiration of the brain with that of other organs. The dark colour of the blood in the cerebral veins suggests fairly considerable consumption of oxygen and the immediate loss of consciousness, produced in man by cessation of the normal supply of arterial blood, shows the dependence on constant supply of oxygen. If the actual consumption is small, this latter fact shows that it must be present with a high tension in order to act efficiently.

In the work of Alexander and Cserna (1913) the rate of flow was determined and the conclusion was arrived at that the small values of Hill and Nabarro were due to the animals being narcotised. If allowed to escape from the influence of deep anæsthesia, the oxygen consumption was found to be very considerable, namely, 0·36 c.c. per gram per minute. If this is not a misprint for 0·036 c.c., it seems to throw some doubt on the accuracy of the method used, since Barcroft and Dixon only obtained 0·011 for the heart muscle and Barcroft 0·028 for the salivary glands (see Barcroft's article, 1908, p. 757).

A. R. Moore (1919) finds production of carbon dioxide as a result of activity in nervous tissue. When extensive degeneration is taking place, products of the breaking down of lecithin, etc., may be found in the blood or cerebro-spinal fluid. Otherwise, the metabolism of nervous tissue is unknown.

THE NERVE NETWORK

It is obvious that there must be physiological continuity between constituent units of the nerve centres in all cases. In all animals above the Cœlenterate condition, however, there is not direct structural protoplasmic continuity between the neurones; we have already spoken of the synaptic membrane intervening, and we shall have to discuss its properties presently. In the Cœlenterates, and possibly in certain parts of the higher animals, there is a kind of nervous tissue, possessing certain properties of central nature, in which a direct anatomical connection appears to exist between the various nerve fibres and cells. This is associated with the power to conduct impulses in all directions, as we saw in the case of the swimming movements of the jelly-fish. Here the ability to perform co-ordinated movements depends upon the refractory phase, so that impulses arriving at the centre when in a state of activity are inoperative. (See Romanes, 1876, and Bethe, 1903, pp. 76-124.) For the "trapped" excitation wave, see A. G. Mayer, 1906, 1908, and Harvey, 1912.

Von Uexküll (1909, p. 81) finds it necessary to introduce the conception of "interrupter" or "representant" as intervening between the nerve net and the muscle fibre, whose property it is to accumulate and give out "excitation." This excitation flows from a place of higher pressure in the network to one of lower pressure, so that a stretched muscle become stimulated and vice versa (see also p. 135 of von Uexküll's book).

In higher invertebrates, such as the earthworm, the neuropile of the central nervous system has been stated to consist of a network of the processes of the afferent and efferent cells. It is a matter of much difficulty to be certain as to whether there is direct anatomical continuity, and, in fact, Retzius (1892, p. 14) says that connection is by contact.

Even in vertebrate animals, we find in peripheral parts nervous interlacings which appear to be networks. A figure of such an arrangement in the palate of the frog is to be found on p. 79 of Bethe's book (1903). Similar structures exist on the walls of blood vessels, and in connection with smooth muscle generally (see Fig. 109, page 402, after Retzius, 1892). With regard to these "networks," although there are local thickenings to be seen in stained preparations, especially at places where branches are given off, there is no evidence that they possess the properties of centres, such as that of reflex action. Moreover, if the figures given by Retzius (1892) be looked at carefully, the impression is given that there is no anastomosis between branches of *different* nerve fibres. At the same time, in such cases as those of the vasomotor nerves, there is no necessity for the separate stimulation of different muscle cells, as in the delicate adjustments of voluntary

muscle; a considerable number of smooth muscle fibres are required to be in action at the same time. But, even if they exist, it is obvious that such networks are not the same thing as the nerve *centres* of the lower invertebrates.

An exception must, perhaps, be made in the case of *Auerbach's plexus* in the alimentary canal. This seems to possess reflex functions as we have seen (page 367), and ganglion cells are readily to be detected in it. This system, however, possesses the properties of a synaptic system, such as excitation and inhibition in definite directions, rather than the indifference of the typical network, and Gaskell has shown that the hypothesis of reflex action is unnecessary (see page 367).

It is of interest in this connection that Meek (1911) has found that the plexus connections are regenerated in about four months after transection of the intestine. The muscular and epithelial regeneration is completed considerably earlier than the capacity of transmitting a wave of inhibition, thus giving additional evidence that the "myenteric reflex" is of nervous origin.

Hofmann (1907), as the result of extensive and detailed investigation of certain *peripheral networks*, especially those in the heart muscle and those innervating the chromatophores of the Cephalopods, comes to the following conclusions, which he finds to agree with the observations made by other workers, with regard to the smooth muscle of vertebrates in general. The nerve bundles supplying such muscular structures form a primary plexus by branching and by division of the coarse nerve fibres contained in them. This plexus is, as regards the direction of its fibres, independent of that of the muscular strands, and often passes transversely across them. These fibres do not anastomose with each other. In fact, Hofmann had previously shown (1904) that each nerve fibre has its own area of distribution and that there is no spreading of excitation, such as we meet with in the Medusæ. From this plexus, again, fibres are given off which form another, "terminal," plexus, whose single fibres run close beside the muscle cells, not forming definite "endings" in or upon them, but finally looping backwards and joining other branches of the same fibre, or perhaps other fibres of the same nerve, although it was not possible to decide whether the latter was really the case and whether a continuous network of the branches of the same nerve bundle may exist. The possibility of fine short fibrils passing to the muscle substance from the loops is not excluded by these results and it does not seem unlikely that there is some further connection between the nerve fibre and the muscle cell than the mere contiguity of the loops. There is nothing of the nature of ganglion cells present in these plexuses. What have been taken for them can be seen in good preparations to be separate from the nerve fibres and appear to be nuclei of connective tissue sheaths to the nerves.

The importance of these facts with respect to the heart will be seen later.

Peripheral networks of the kind described above clearly serve only for conduction and not for origination of nervous impulses, nor as reflex centres.

Owing to the fact that nerve fibres conduct in both directions, it will be seen that, if one branch of a nerve fibre, which has divided, is put into excitation by some means, the impulses will spread over all the other branches of the same fibre and to any other fibres with which these may be connected in a network. We have, in such a case, an "*axon reflex*," as defined by Langley (see page 425). Excitation must spread to any effector cells supplied by any of the fibres. I refer to this fact here on account of certain curious phenomena connected with the *vaso-dilator mechanism of the skin*. When the peripheral ends of dorsal roots are stimulated, the region which has its sensory innervation in the particular root stimulated shows that its arterioles have dilated. Doi (1920) shows that the phenomenon is present in the frog, and that the dilator effect is exercised both on arterioles and on capillaries. I was able to show (1901, 2) that the nerve fibres concerned have the same kind of anatomical connections, and show the same degeneration relations, as the ordinary afferent fibres. It is well known that certain substances, such as mustard, placed on the skin, produce inflammation there. Spiess (1906) observed that such inflammation does not occur if the area of skin is anæsthetised, as by cocaine, and thought that the inflammatory process was brought about by a reflex from the spinal cord to vaso-dilator nerves. Ninian Bruce (1910), however, found that the inflammation was still produced

after mere section of the dorsal roots, so that it could not be a spinal reflex. It was not present, on the other hand, if sufficient time had been allowed for the nerve fibres to degenerate. The vaso-dilatation produced by mustard depends on the integrity of sensory nerve fibres, although not an ordinary reflex. There seems to be no other explanation except that given by Bruce himself, namely, that the phenomenon is of the nature of an axon reflex. The sensory fibres must be supposed to branch at the periphery, one part supplying receptors in the skin, the other supplying the muscular coat of arterioles and acting there as efferent inhibitory fibres (Fig. 145). When the receptor branch is stimulated, excitation spreads to the main fibre, and back along the vascular branch to the blood vessels. It may be that this latter enters a peripheral network, as suggested by me (1901, 2, p. 196).

THE SYNAPTIC SYSTEM

The anatomical unit of the higher central nervous systems is, as we have seen, the neurone. Perhaps the clearest proof of the *structural* discontinuity of the individual neurones is afforded by the fact that the degeneration which takes place in a nerve fibre, when it is cut off from the rest of its neurone, only proceeds as far as its contact (synapse) with the processes (dendrites) of another neurone. Although physiological continuity must exist, there is evidently an absence of protoplasmic or nutritive continuity. As Sherrington points out (1906, p. 17), such a contact surface is of great functional importance since "it might restrain diffusion, back up osmotic pressure, restrict the movement of ions, accumulate electric charges, support a double electric layer, alter in shape and surface tension with changes in difference of potential, alter in difference of potential with changes in surface tension or in shape, or intervene as a membrane between dilute solutions of electrolytes of different concentration or colloidal suspensions with different sign of charge"; in fact, all the numerous phenomena which the earlier chapters of the present book have shown to be of fundamental importance in the mechanism of the cell. We have also manifold possibilities of excitation and inhibition in the use of one and the same neurone in different nervous acts and the consequent advantages to the organism in economy of machinery.

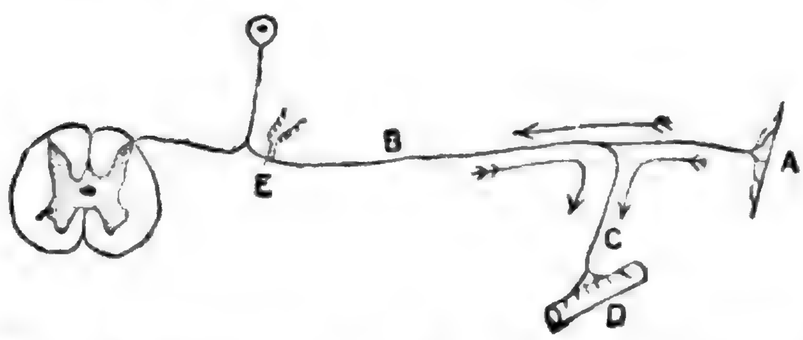

FIG. 145. DIAGRAM OF REFLEX ANTIDROMIC VASCULAR DILATATION AS AXON REFLEX.

A stimulus at *A* gives rise to an impulse passing along fibre *B* to the spinal cord. A branch from this fibre *A* is given off at *C*, which ends in the walls of the arteriole *D*. Stimulation of the sensory end organ of the fibre at *A* gives rise to an impulse which passes to the arteriole along the branch *C* in addition to reaching the spinal cord, if the main fibre *B* is intact.

(Bainbridge and Menzies, "Essentials of Physiology," p. 289.)

Some points regarding the properties of the synaptic membrane have been already alluded to, but may be recapitulated here.

Since there is no structural continuity, the possibility of actual retraction owing to increase of surface tension must be admitted.

The action of electrolytes (page 218), chloroform, and strychnine (page 427) is, no doubt, exercised on the synaptic membrane, which, like other cell membranes, is, presumably, a colloidal system.

The *summation* of a series of ineffective stimuli, so that a reflex is ultimately produced, is a common property of nerve centres. Also "facilitation," as it is called by Sherrington, in which an effective stimulus leaves the mechanism for a time capable of excitation by stimuli which were previously too weak, seems to be a further aspect of the same phenomenon. The work of Adrian and Lucas (1912, p. 121) has been already referred to.

Adrian's work (1912, p. 411) on the "*all or nothing*" principle has important

consequences for the central nervous system, since it shows that a disturbance cannot be permanently altered in strength by passing through some region of decrement. We cannot assume that it can be made in this way too small to pass through the synapse, which must itself be looked upon as a place of decrement (see page 426). This fact shows the importance of the actual connections of a particular neurone; in other words, the anatomy of tracts and the centres which they bring into relation with one another is of essential importance. At the same time, the effect of strychnine shows that, in the spinal cord, there is potential communication, at the least, between a receptor and all the motor neurones. A localised stimulus sets into activity the whole of the muscles of the body.

Irreciprocal Conduction.—It seems to be a very usual property of the synaptic membrane to allow impulses to pass in one direction only. Thus Gotch and Horsley (1891, p. 485) found that stimulation of the central end of an efferent root caused no electrical change in the spinal cord above, although that of an afferent root did so. On the other hand, the discharge of a spinal centre flows, in part, *backwards* down the other afferent, dorsal roots. Vészi (1909), by an ingenious form of experiment, has shown that continued stimulation of a motor nerve produces no fatigue in the reflex centres; the excitatory process does not spread inwards as far as the place where central fatigue occurs. Some further facts will be found in the next chapter (page 491).

We have seen (page 141) how permeability of a membrane to one only of the ions of a salt may allow an electrical current to pass in one direction only. Irreciprocal permeability is, therefore, a state experimentally realisable.

Fatigue.—We have already seen that a motor centre may be fatigued for one reflex but remain unaffected for another (page 423). This state of fatigue is, accordingly, situated in some *synapses*, not in the efferent neurone itself. Excessive fatigue has been found to result in changes in the cell substance, as the experiments of Dolley, referred to on page 16 above, and of other observers, show.

REFLEX ACTION

In the most primitive condition, an effector may be excited directly by a receptor cell, or its prolongation, as in the sea anemone. But this arrangement cannot be called a central nervous system. Although the neurone is the anatomical unit of such systems, the reflex is the functional unit, having as its anatomical basis the reflex arc. This, in its simplest possible form, consists of at least two neurones in addition to the effector cell itself. The receptor neurone forms a synapse with the motor neurone, whose cell body is in the central nervous mass and whose axon passes out to excite an effector. Put in another way, the mechanism consists of three parts, receptive, conductive (including nerve fibre and central cell), and effective (the peripheral organ set in action). Even in the vertebrate, the cell body of the receptor neurone, although moved up to the dorsal root ganglion, as stated above, is still outside the central nervous system. But there is, in any central nervous system, except the very simplest, a synapse between at least two neurones. The economy, as well as the integrative efficiency, resulting from the use of one motor neurone by several receptors could not otherwise be obtained. This is the principle which is called by Sherrington (1906, p. 55) that of the "*final common path.*"

The simplest kind of reflex arc is to be found in the stellar ganglion of the Cephalopod, according to the work of Fröhlich (1909, 1). That this ganglion has central functions is shown by the fact that stimulation of a point of the mantle causes contraction over a wide area if the ganglion is intact, but if it is removed, contraction is limited to the spot stimulated. The application of strychnine has no effect, hence the conclusion is drawn that the intermediate neurone, or its synapse, on which this alkaloid acts, is absent and the arc consists only of the receptor neurone forming a synapse with the motor neurone in the ganglion. That is, there are two neurones and one synapse.

According to the experiments of Jolly (1910), the "*knee-jerk,*" that is, the contraction of the extensor muscles of the knee evoked by tapping the tendon

below the patella, has a "synapse time" of 0·002 second, while that of the flexion reflex is 0·004 second. If the latter involves two synapses and three neurones, it appears that the knee-jerk only involves one synapse and two neurones, an afferent one from the tendon, and the motor neurone to the muscle fibre. The experiments were made by the use of the string galvanometer. By this means it is possible to record electrical changes in the afferent fibres and in the efferent fibres, when the tendon is struck, and the various times making up the total latency can be determined. The knee-jerk must, perhaps, be regarded as an exceptional form of reflex in the higher vertebrates, although it shows the possibility of an arc of two neurones only. The following measurements from Jolly's paper are of interest:—

	Knee-jerk.	Flexion Reflex.
Total latency	0·0055	0·0106
Afferent endings	0·0005	0·0028
Conduction time	0·0014	0·0020
Motor endings	0·0015	0·0015
Synapse time	0·0021	0·0043

Subtracting the sum of the second, third, and fourth numbers from the total latency, we have the time spent in passing synapses, as given in the last line.

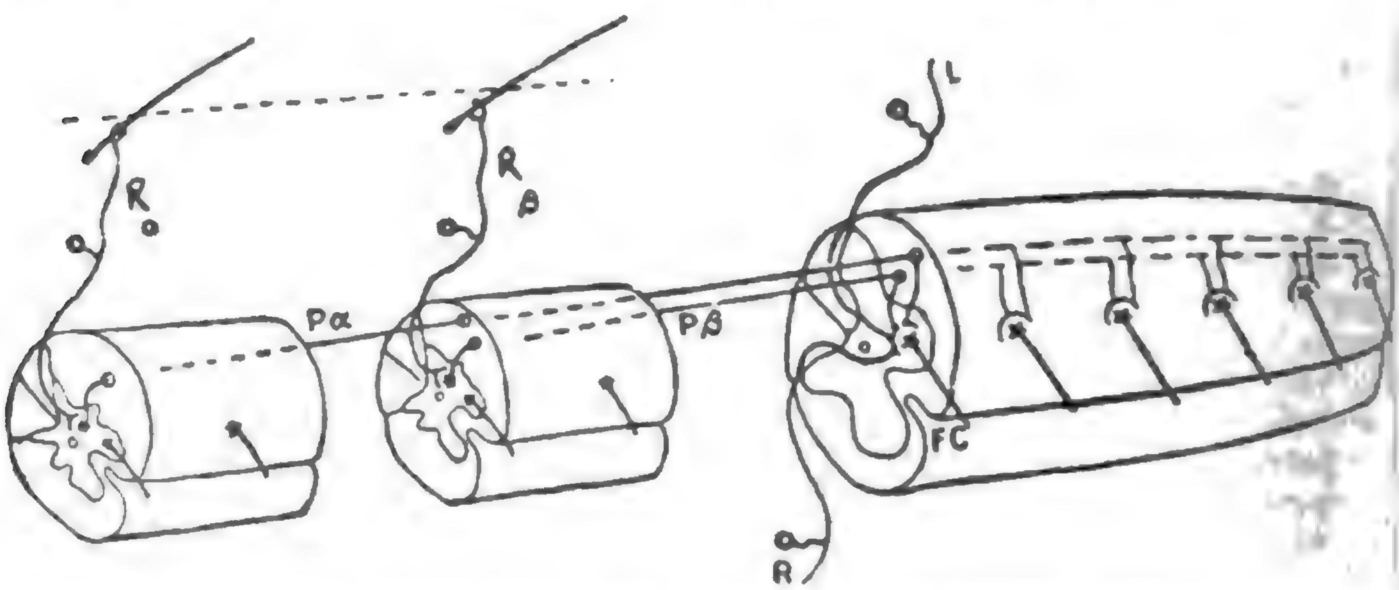

FIG. 146. DIAGRAM OF THE SPINAL ARCS INVOLVED IN THE SCRATCH REFLEX IN THE DOG.

L, Receptive or afferent nerve path from the left foot.
R, Receptive nerve path from the right foot.
Rα, *Rβ*, Receptive nerve paths from hairs in different regions of the dorsal skin of the left side.
FC, The final common path, in this case the motor neurone to a flexor muscle of the hip.
Pα, *Pβ*, Proprio-spinal neurones.

(Sherrington, 1906, p. 119.)

In actual experience, there is usually to be found at least one additional, intermediate neurone, the whole of which is contained within the nerve centre. Thus the "scratch reflex" of the dog consists of the following elements (Sherrington, 1906, p. 54), (Fig. 146).

1. The receptor neurone from the skin of the back to the grey matter of a certain spinal segment in the shoulder region. This forms a synapse in the grey matter with

2. A long neurone in the spinal cord itself (proprio-spinal), which passes backwards to the segments of the hind leg. Here it forms a synapse with

3. The motor neurone, whose axon supplies a flexor muscle of the leg performing the scratching movement.

There are thus three neurones and two synapses. There may, indeed, be more

intraspinal neurones and synapses between (1) and (2) and between (2) and (3), but the arrangement described is the simplest possible for such a reflex as the one in question, having its receptors and effectors in parts of the body distant from one another.

The motor neurone (3) is the *final common path*, and the rest of the arc up to it from the receptor is the *afferent arc.*

The complete mechanism of the scratch reflex includes, of course, also a cutaneous sense organ, which intensifies such a stimulus as the bite of a flea, for example, so as to produce a propagated disturbance in the receptor neurone; and at the other end, the fibres of the flexor muscle, the actual effector, are a part of the mechanism.

There are some important general characteristics of reflex action which will best be deferred till the following chapter.

FUNCTIONS OF THE "BRAIN"

The increase in complexity and effectiveness of the central mechanism, due to the addition of longer intermediate neurones, as well as the addition of more and more intermediate neurones to form cross connections, has been referred to above. At the anterior end of an animal, we find the gradual evolution of the "*head*" with its specialised and elaborate receptor system, notably that of the "distance receptors," as they are called by Sherrington. These enable the organism to be affected by occurrences which do not themselves come into actual contact with it; such occurrences are those affecting organs sensitive to light or to sound.

In connection with these distance receptors, the highest part of the central nervous system, the "*brain*," is developed. This part of the system has control over all the rest. In ourselves, we know that it is associated, in some way, with consciousness.

So far is it the case that the brain is to be regarded as the central ganglion of the distance receptors, that, as Langendorff has pointed out (Sherrington, 1906, p. 349), a blinded frog is like one with its cerebral hemispheres removed; a shark without olfactory lobes behaves as if it had lost its fore-brain.

Now Pavlov has worked out a method, that of "*conditioned reflexes*," to be described in the next chapter, by which it is possible to investigate the mechanism of the highest centres without appeal to consciousness. Results of great value are to be obtained by this method. Pavlov himself regards the introduction of psychological modes of expression as obscuring the problems. No doubt, the physiologist is not, as such, concerned with phenomena of consciousness, and the introduction of a method in which they can be omitted is a great advance. At the same time, the phenomena of the higher sense organs necessitate the use of methods of investigation involving the introduction of consciousness, although it is used as an indicator only.

The methods of comparative psychology may also be referred to as illustrating the possibility of investigation of the higher functions of the nervous system without the necessary introduction of consciousness. The book by Margaret Washburn on "The Animal Mind" (1909) may be consulted by those interested.

A description, in any detail, of the functions of the higher parts of the central nervous system would require much more space than can be given in a book with the scope of the present one. Fig. 147, compared with Fig. 143 (page 468), may serve to give some idea of the kind of connections that are found in the higher nerve centres. There are, however, a few facts of general interest that may with advantage be given here.

Those functions which we know as "mental" have their seat in the cerebrum, especially in the cortex or pallium, if the philosophers will pardon the use of the word "seat" in this connection. It is, then, at first sight, a somewhat surprising fact that, by stimulation of certain limited regions of the cortex, definite, localised movements of limbs, face, trunk and so on can be evoked. The area which has these properties is known, for convenience, as the "*motor area.*" Arising from this we have the system of long neurones known as the "pyramidal tract," con-

sisting of the axons of giant pyramid cells, the "Betz cells," of a particular layer in the grey matter of the cortex. These axons form synapses with certain inter-

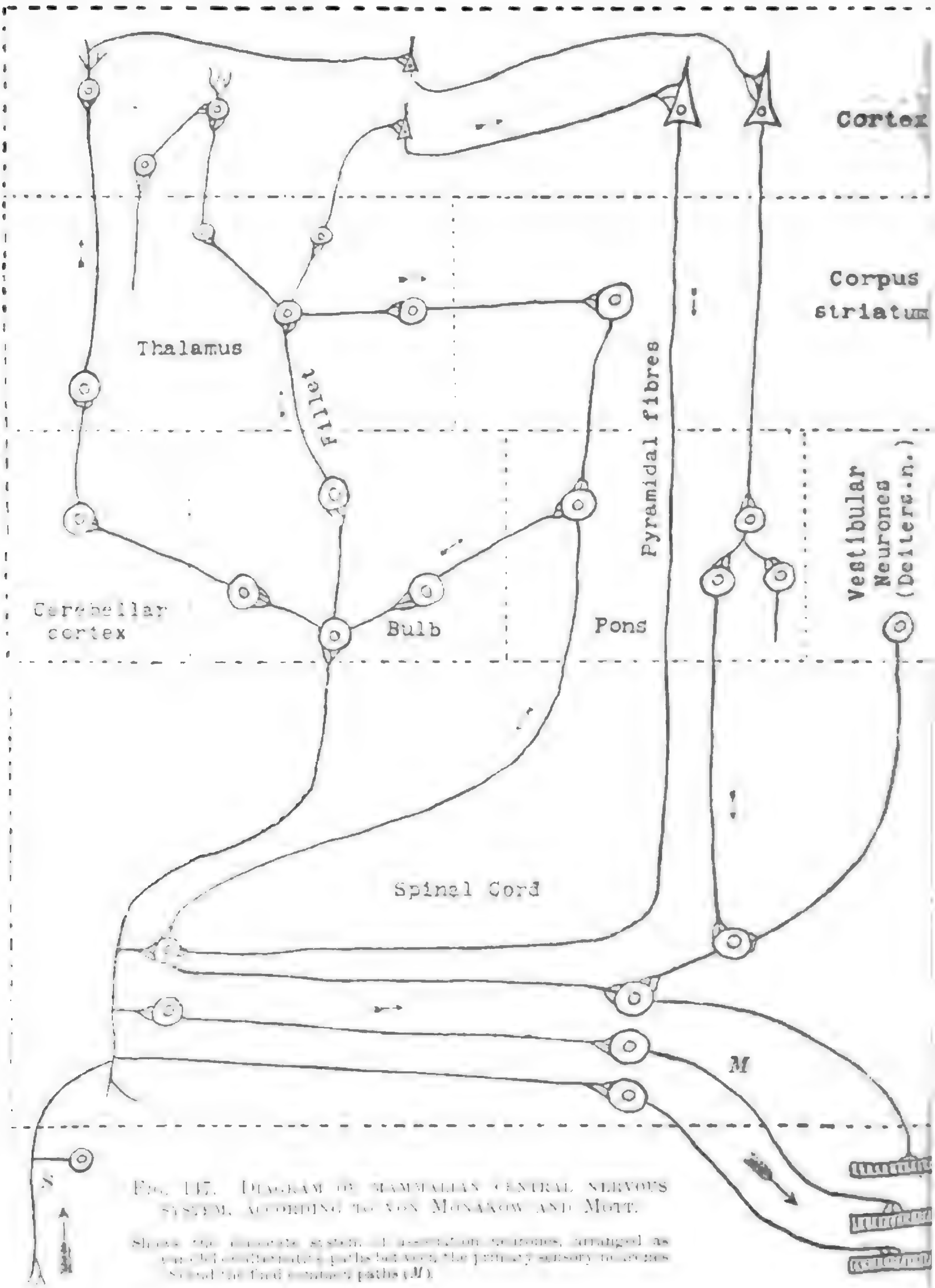

FIG. 147. DIAGRAM OF MAMMALIAN CENTRAL NERVOUS SYSTEM, ACCORDING TO VON MONAKOW AND MOTT.

Shows the complex system of association neurones, arranged as parallel conductors between the primary sensory neurones (S) and the final common paths (M).

mediate neurones as far away as the distant end of the spinal cord and ultimately with the motor neurones of the final common path. These Betz cells are found to disappear when the axons forming the pyramidal tract are cut through. Fig. 148 (Holmes and May, 1909) shows this fact. The view that the pyramidal fibres do

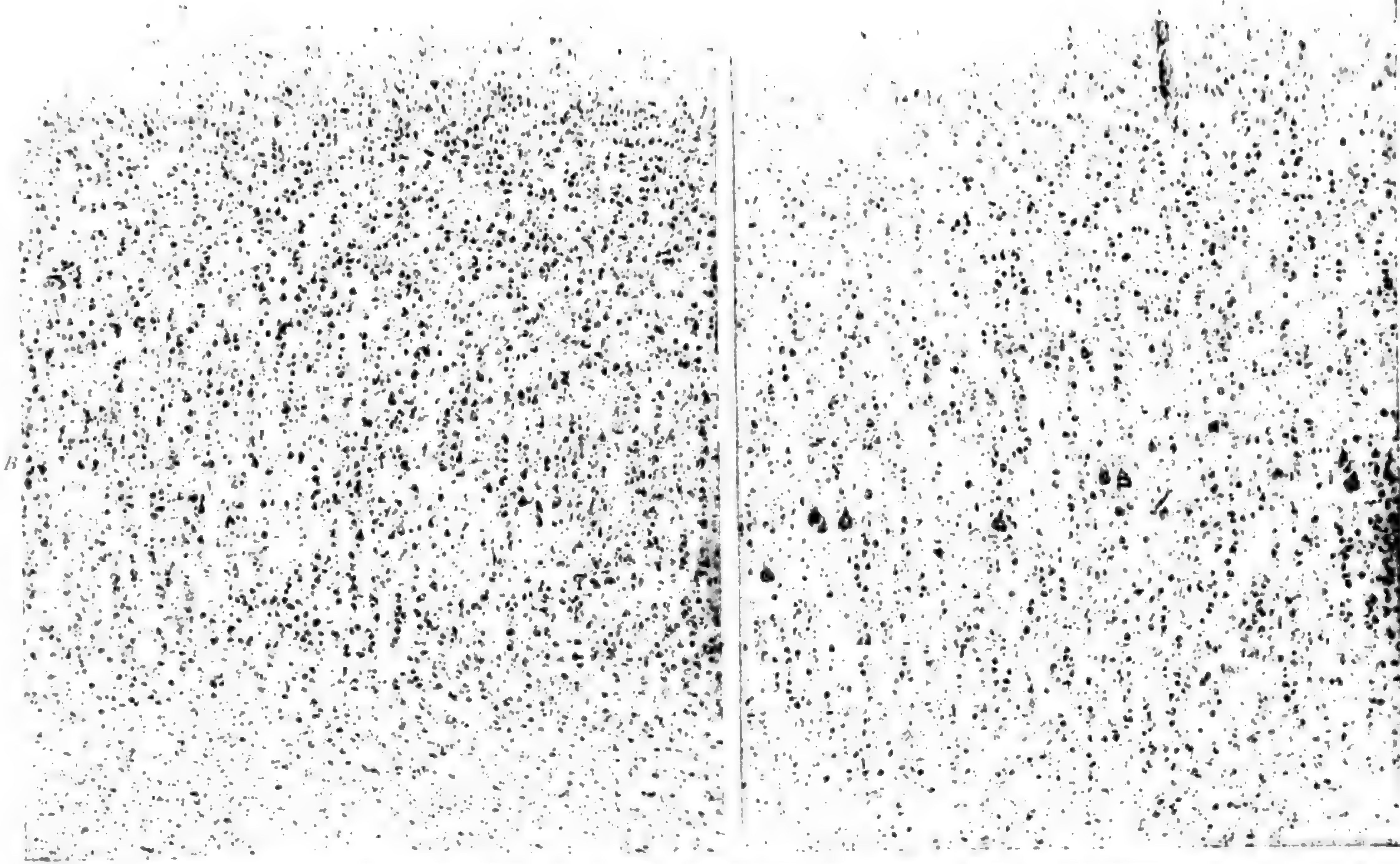

FIG. 148. TO SHOW THE ORIGIN OF THE PYRAMIDAL TRACT FROM THE GIANT CELLS OF BETZ IN THE CEREBRAL CORTEX.

A, Photomicrograph of the normal precentral cortex of the macaque monkey. Note the row of giant cells just below the middle.

B, From a section in a corresponding part of the left hemisphere 157 days after division of the right side of the spinal cord. Only the indistinct remnants of two giant cells are visible. Owing to the section of the axons of these cells in the spinal cord, the cell bodies undergo degeneration and can no longer be rendered visible by staining.

not form synapses directly with the motor neurones of the cord, as was supposed at one time, but with intermediate neurones more on the afferent side, appears to be favoured by most neurologists at the present time. It should also be mentioned that the motor area has a different histological structure from that of other, non-excitable regions.

The first definite proof that movements are evoked by electrical stimulation of particular regions of the cortex was given by Fritsch and Hitzig (1870) and was a very important advance in knowledge. It was supposed by many that "mental" functions were independent of the material constitution of the nervous system and insufficient credit is given to Gall (1825, Gall and Spurzheim, 1810-1819) for having propounded a more scientific view. It is true that his system was based on very superficial considerations, but it was Auguste Comte (1877, 3, 565-570) who first drew attention to the philosophical importance of his work.

When we call the area of the cortex from which movements can be excited, the motor area, it is not to be supposed that we use the words in the same sense as when applied to spinal motor neurones in the ventral horn of the grey matter. What we stimulate in the former case appears to be some part of a certain complex system of neurones, the activity of which implies a particular movement. So that, if we regard all that part of a complex arc up to the final motor neurone as belonging to the afferent side, we may speak of these cortical areas as "*sensori-motor*" or "*kinæsthetic*" in accordance with the view of Bastian (see his book of 1880, pp. 584-588).

The work of Graham Brown and Sherrington (1913) shows that destruction of the motor cortex does not produce permanent paralysis of even delicate voluntary movements of the part whose "area" has been removed, even in an animal as high as the chimpanzee. The arm area on the left side was removed, with the usual result of paralysis of the right arm. In the course of four and a half months, recovery was so complete that no difference could be detected in the behaviour of the two arms. Now there are three explanations that might be suggested for the recovery.

1. Regeneration of the area destroyed. This is excluded by the fact that, six and a half months after the first operation, another operation was performed and the area in question was found to be completely inexcitable.

2. Taking over of the movements of both arms by the corresponding area on the normal side of the cortex. To test this, four and a half months after the first operation, the arm area was destroyed on the *right* side. Although the immediate result of this was paralysis of the left arm, there was no change in the movements of the right arm, which had recovered from the previous paralysis. In two months more, complete recovery of *both* arms had taken place.

3. The post-central convolution, itself not motor, that is, not excitable by electrical stimulation, might have taken over the function of the arm area immediately in front of it. Two months after the second operation, this convolution was removed. At the operation it was found to be, as usual, inexcitable and its removal did not cause paralysis of voluntary movement, although for two or three weeks after the removal there was weakness in some movements, but this completely disappeared later.

The reactions to be obtained by stimulation of the motor cortex are, compared with those of spinal reflexes, much more modified by slight variations in the condition of the animal, blood supply, narcosis, etc. A systematic investigation of the reaction to be obtained by electrical *stimulation of cortical points* was made by Graham Brown and Sherrington (1912). They took two points, one giving primary flexion at the elbow, the other primary extension. The two antagonistic muscles, supinator longus and the humeral head of the triceps, were connected to levers for tracing. The effects obtained were very complex. Variable latency, various after-actions, such as rebound, tonic and clonic, mutual relations of great diversity as regards the pair of antagonists, show the high complexity of cortical reactions. Inhibition appears more prominent than excitation and seems to be independent of simultaneous excitation of the antagonist muscle, thus differing from typical reciprocal innervation of spinal reflexes to

be described in the next chapter. The same cortical point, after rest, yields very nearly the same result as it did on previous occasions; but, if it be stimulated immediately after a previous response, the result is usually found to be reversed; that is, a point giving excitation after rest gives inhibition if stimulated again after an excitatory response. Suppose, again, that a point gives extension of the elbow, and that then another point which gives flexion of the elbow is stimulated and finally stimulation of the extension point is repeated. It is usually found that the effect is reversed, giving flexion. In the decerebrate preparation, stimulation of an afferent nerve of the limb observed causes contraction of the flexors and inhibition of the extensors. With the cerebrum intact, the action of the cortical flexion point is augmented by stimulation of such an afferent nerve, and the effect of a cortical extension point is reversed to flexion; so that, if the latter point were being stimulated and giving its normal extension effect, stimulation of the afferent nerve may reverse the effect to flexion, but the result depends much on the relative strengths of the two stimulations. If two antagonistic cortical points are stimulated, there is some indication of algebraical summation of the opposed actions. The general conclusion is drawn that one of the special functions of the cortex is to reverse the factors of purely spinal or decerebrate reflexes, when necessary.

In connection with these results, the work of Osborne and Kilvington (1910) is of interest. One of the nerve cords of the *left* brachial plexus was cut, and its central stump joined to the peripheral stump of the corresponding cord of the *right* side. After time for regeneration, ten months, stimulation of the *right* motor area gave movements of *both* paws, although normally it gives movements of the left only. The left motor area was dead. A point of importance is that the natural co-ordinated movements of the limbs appeared to be quite normal, so that the conclusion is justified that the motor centres of the cortex can change their function. The part of the left motor area in the above experiment must have been assumed by that of the right side. Kennedy (1914) performed similar experiments. He joined together the nerves of the fore limb of the dog in such a way that both extensors and flexors were supplied by the same nerve, while the antagonist nerve was eliminated. After regeneration, the respective cortical centres were stimulated. That of the eliminated nerve was inexcitable, according to the usual rule. The other centre, which would normally have produced either flexion or extension only, according to the point excited, caused contraction of *both* antagonists, and at no part of the centre could contraction of either group alone be obtained.

Some experiments by Burnett (1912) serve to illustrate further points in the function of the cortex. The behaviour of frogs from which the cerebral hemispheres had been removed was much more machine-like and predictable than that of normal ones, although, on casual observation, there was not much difference to be detected, so long as they were not exposed to any new conditions. The normal and the decerebrate frogs were kept together in the same vivarium, and if flies were put in, the normal frogs were more skilful and accurate in capturing them. On the other hand, if a frog of each kind was removed, placed under a glass jar on the table, and flies added, the decerebrate frog captured them all in a few minutes, while the normal frog spent all his time in trying to escape from the holder or in a crouching position, apparently inhibited by fear.

The various analysing mechanisms connected with sense receptors have also been localised to a considerable degree. Details are beyond the space available in these pages.

A valuable general discussion of cerebral localisation will be found in the paper by Graham Brown (1916).

THE CEREBELLUM

The best general statement of the functions of the cortex of the cerebellum is that contained in the view of Sherrington (1906, pp. 347-349), namely, that it is the supreme ganglion of the proprio-ceptor system. It co-ordinates all movements in relation to normal posture, in response to information received, not only from the muscles themselves, but also from the labyrinth. Its intimate relation with Deiters' nucleus, the centre of the labyrinth nerves, and with the cerebral motor

cortex is explicable on these lines. We also understand why the cerebellum is concerned with decerebrate tonus. This view has been found by Gordon Holmes so well adapted to explain the results of injuries to the cerebellum as to receive important confirmation (see Holmes, 1918).

No reaction is to be obtained from electrical stimulation of the cerebellar cortex. Edinger had already suggested that it is a chief sensory centre, and the work of Horsley and Clarke (1908) was in agreement therewith.

MEMORY AND ASSOCIATION

Since no fresh neurones are formed during the life of an animal, and when the cell body of a neurone is destroyed no regeneration occurs, it will be obvious that any new acquirement in reflex or association must be due to the formation of new connections between neurones already present. Memory thus implies the more or less permanent establishment of these connections. The possibility of disconnection at a later period must clearly be admitted.

It appears that, in the lower organisms, such as insects, a habit may be formed by long training; so that, for example, they may become able to find their way to food by a complex path. But suppose that the arrangement is altered back to the simple one for a time and then the complex one, to which the new adjustment has been formed, is returned to. It is clear that the length of time the new acquirement lasts can be tested; and experiments have been made on the cockroach which show that about half an hour is the length of time during which this animal is able to remember what it has learned.

In the higher animals, new associations are formed, so far as we know, only in the cerebral cortex. The experiments of Burnett (1912), already referred to, showed that decerebrate frogs were unable to form even the simplest associations.

In the lower animals there appears to be less centralisation. Yerkes (1912) found that an earthworm, which had been caused to form a habit of taking a particular course, did not lose the "memory" when the cerebral ganglia were removed.

With regard to the gratuitous introduction of such expressions as judgment, or decision by some sort of a controlling "mind," which it has been thought by some to be necessary to introduce even into the interpretation of the phenomena shown by some of the simplest nervous systems, the experiments of A. A. Moore (1910) on the starfish are to the point. The central nervous system of this organism is in the form of a ring, from which a nerve passes radially to each arm. If a simple cut be made across this ring, no break is made in the actual possibility of control of each arm by the centre. The fact that the arm next the cut does not co-ordinate with the others in the righting movement proves that direct nervous connection across the place cut is necessary for "intelligent" co-operation. Any one arm can initiate impulses which affect strongly only adjacent arms and rapidly decrease as they travel from their point of origin. Yet this simple mechanism is sufficient to account for the complicated righting movements of the animal (see also A. R. Moore, 1920).

The paper by Carveth Read (1911) may be referred to in connection with the relations between instinct and intelligence.

SPEECH

No reference has been made as yet to the nervous mechanism of speech and those other powers, such as reading and writing, which depend upon it.

Early in the evolution of social communities we find means of some sort for the purpose of communication of signals of danger and so on. But very little is possible with inarticulate sounds and it is only when articulate speech, with a great variety of words having definite meanings, commenced that mental evolution made rapid strides.

For the cerebral centres and connections involved, the reader must be referred to the textbooks of Human Physiology and especially to the monograph by Mott (1910).

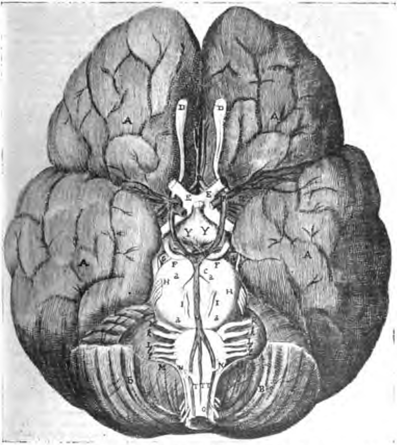

FIG. 149. WILLIS' PLATE OF THE BASE OF THE HUMAN BRAIN, SHOWING THE CRANIAL NERVES AND THE CIRCLE OF WILLIS.

The following is the description given by Willis :—

A, A, A, A,	Cerebri quadripartiti anteriores posterioresque, lobi.
B, B,	Cerebellum.
C, C,	Medulla oblongata.
D, D,	Nervi olfactorii, sive par primum.
E, E,	Nervi optici, sive par secundum.
F, F,	Nervi oculorum motorii, sive par tertium.
G, G,	Nervi oculorum pathetici, sive par quartum.
H, H,	Nervorum par quintum.
I, I,	Nervorum par sextum.
K, K, K, K,	Nervi auditorii, et eorum utrinque bini processus, par septimum.
L, L, l, l, l, etc.,	Par vagum, sive octavum, pluribus fibris constans.
M, M,	Nervus spinalis, ad originem paris vagi à longinquo accedens.
N, N,	Par nonum, pluribus etiam fibris constans (quæ deorsum tendentes, in eundem truncum coalescunt) qui paulo supra processum occipitis emergit.
O, O,	Par decimum deorsum tendens.
P, P,	Arteria carotidis truncus abscissus, ubi in ramum anteriorem et posteriorem dividitur.
Q, Q,	Ejus ramus inter duos cerebri lobos incedens.
R,	Carotidum rami anteriores uniti abscedunt, in cerebri fissuram pergentes.
S,	Carotidum rami posteriores uniti, et trunco vertebrali occurrentes.
T, T, T,	Arteriæ vertebrales, et earum tres rami ascendentes.
V,	Vertebralium rami in eundem truncum coalescentes.
W, W,	Locus designatur, ubi arteriæ vertebrales et carotides uniuntur, et utrinque ramus ad plexum choroïdem ascendit.
X,	Infundibulum.
Y, Y,	Duæ glandulæ pone infundibulum consitæ.
a, a, a, a,	Protuberantia annularis quæ à cerebello dimissa medulla oblongata caudicem amplectitur.

(Willis, 1680, Pl. 1. Drawn by Christopher Wren. See text, page 484.

METHOD OF INVESTIGATIONS

In general terms, these methods have been described incidentally in the preceding pages. They may be broadly divided into those of stimulation and those of destruction of localised areas. The various histological methods of staining different kinds of tissue and of degenerated tracts are also of importance.

For accurate stimulation or destruction of localised spots in the interior of the brain, the "stereotaxic instrument" of R. H. Clarke (Horsley and Clarke, 1908, pp. 19-39) is of great value. This has been recently improved, but details of the latest form of the instrument have not yet been published.

The strychnine method of Dusser de Barenne (1916) was found useful by him in sensory localisation.

THE CEREBRAL CIRCULATION

Before passing on to the consideration of certain questions relating to the innervation of the viscera and the blood vessels, a few words may be said as to the blood supply of the brain. As will be seen later, there is no adequate evidence that the cerebral vessels have any vasomotor control; the importance of the brain is such that its circulation is regulated by the whole of the rest of the body, which is caused to accommodate itself, by constrictor and dilator nerves, to the needs of the brain (Bayliss and Hill, 1895).

A further interesting fact is the way in which, in the higher vertebrates, the main arterial supply is formed by cross connections between all the four arteries taking part, so that the interruption of one source does not deprive the brain of blood. This arrangement, known as the "circle of Willis," is shown in Fig. 149, which is a copy of one of Willis' plates (1680).

This plate is of interest for two other reasons. It shows the numeration of the cranial nerves with which the name of Willis is associated, and it was drawn for him by his friend, Christopher Wren, who, as is said in the preface, "eruditissimis suis manibus delineare non fuit gravatus," "did not think it too much trouble to draw with his skilful hands" many of the plates in the book.

THE INVOLUNTARY, OR "AUTONOMIC" NERVOUS SYSTEM

The relation of the nervous supply of the viscera to that of the muscular and other skeletal (somatic) components of the organism was first made clear by the work of Gaskell (1886 and 1889) to which that of Langley (1891 and onwards) added important extensions. Gaskell's work on the innervation of the heart led him to see that the sympathetic nervous system, which consists of a chain of ganglia united by nerves with a particular region of the spinal cord, is not a separate nervous system, interchanging fibres with the cerebro-spinal system, as had been taught, but is made up of efferent fibres given off by the thoracic and upper lumbar segments of the cord. These fibres are fine and medullated, being known as the white rami communicantes. The grey rami were shown by Gaskell to be in reality peripheral nerves, forming the axons of certain neurones whose cell bodies are in the sympathetic ganglia. Their distribution is to the blood vessels of the cord and of its membranes. The white rami, then, form synapses in the sympathetic ganglia, or sometimes more peripherally, with neurones whose axons are distributed to viscera, blood vessels and other organs, which are not under voluntary control but can be acted upon reflexly.

According to a remark made by a "philosophic physiologist" (quoted by Cl. Bernard, "Science expérimentale," p. 155), nature thought it prudent to remove these important phenomena from the caprice of an ignorant will.

The white rami of the sympathetic, thus, are identical in nature with what we have called in previous pages, "association fibres." In other words, they are efferent nerve tracts connecting one part of the central nervous system with another. The only difference from those in the voluntary system is that the cell bodies, or centra, with which the latter form synapses, lie within the cerebro-spinal system, while those of the involuntary system pass out before meeting

with the neurones with which they are to form synapses. These latter neurones, accordingly, correspond in nature with the motor neurones of the anterior horn of the cord, called by Sherrington the "final common path." They differ, however, since the axons of the sympathetic neurones, the "post-ganglionic fibres," are non-medullated.

Further investigation, with this clue, led to the recognition of two other similar outflows of efferent, ganglionated, visceral nerves, one in the sacral region, the other in the cranial nerves. These three outflows are separated by two gaps, where the nerve plexuses for the anterior and posterior limbs are found.

The whole system is in no sense an independent central nervous system, but an outflow from the cerebro-spinal system, distinguished by its connection with neurones lying entirely outside the latter, together with its formation of peripheral plexuses at the places of its distribution. We may note that it is entirely efferent. Sensory fibres found in some of its nerve trunks, such as the splanchnics, are ordinary afferent fibres, having their trophic centres in the dorsal root ganglia, and merely taking their course in the sympathetic nerves as sympathetic fibres are distributed in nerve trunks of the voluntary system.

Langley (1898, p. 241), in order to obviate the confusion which might arise from calling the sympathetic nerves which supply the skin, "visceral," proposed the name "autonomic," suggested to him by Professor Jebb. "The word implies a certain degree of independent action, but exercised under the control of a higher power." "The autonomic nervous system means the nervous system of the glands and of the involuntary muscles; it governs the 'organic' functions of the body."

It is necessary to be quite clear that "autonomic" is a name for the *whole* of the involuntary nervous system, *including* the sympathetic, since some writers abroad have used the name as applying to that part of the system which is not sympathetic. For this, the name "para-sympathetic" is used by some; it has its justification in certain peculiarities, which will be described in Chapter XXIV., distinguishing the sympathetic from other parts of the system. Gaskell prefers the name "enteral" for the non-sympathetic part (1916, p. 151).

Although the name "autonomic" is a convenient one and has come into general use, objection might, perhaps, be taken to it on the ground that the involuntary system has no independent action at all, so that the word tends to perpetuate in some degree the old view of the sympathetic ganglia as nerve centres.

The details of the anatomical arrangement of this system must be obtained from the monograph by Gaskell (1916) and the article by Langley (1900). Some of the salient points only can be referred to here.

As Gaskell points out (1916, p. 150), the unstriped muscles of the vertebrate may be divided into groups characterised by their innervation and other properties:—1. Vascular. 2. Those belonging to the skin. 3. Those under the surface of the gut. 4. Those around the segmental duct. 5. The sphincters of the gut. 6. Those connected with the adjustment of vision. It is found that 1, 2, 4 and 5 receive their motor innervation from the sympathetic and react to adrenaline, while 3 reacts to acetyl-choline and is supplied by the bulbo-sacral outflow as regards its motor nerves. The sympathetic supplies all the vaso-constrictor nerves of the body, wherever they are found, and the accelerators to the heart, together with inhibitory nerves to the intestine, secretory nerves to epidermal glands, belonging to group 2. Glands belonging to the endodermal system, gastric and pancreatic, as also the muscles of group 3, are supplied by the enteral system, the bulbar (vagus) outflow, in fact. At the two extremities of the alimentary canal, the two sets of gland cells are mixed, as in the salivary glands, so that these organs are supplied both by the sympathetic and the enteral nervous outflows. The bulbar and the sacral outflows have in common the fact that, unlike the sympathetic, the connectors, or association fibres, do not form synapses with the neurones of their final distribution until this is reached. A well-known case is that of Auerbach's plexus in the distribution of the vagus to the intestine. Similar plexuses exist in the bladder and other urogenital organs in the case of the pelvic nerves.

Auerbach's plexus is developed as an outgrowth from the central nervous system, and is not to be regarded as a survival of a primitive peripheral nervous system (see page 367 and the paper by Miss Abel, 1909).

SUMMARY

The object of a nervous system is to bring any part of an organism into relation with any other part, without the necessity of direct nervous connections from every part to every other part. It is like a telephone exchange, where each subscriber has a central terminal, which can be put into connection with that of any other subscriber. In the nervous system, however, the channels which bring in messages from parts of the body (afferent fibres, coming from sense organs) are, as a general rule, different from those fibres (efferent) which convey messages outwards to organs in the body, which organs are thus caused to perform some kind of action (effectors). As Pavlov has pointed out, something must be sacrificed in the telephone system, since the same subscriber cannot speak to more than one other subscriber at the same time. The arrangements of the central nervous system are more efficient than this, since the same afferent fibre can at times be connected up with several efferent fibres.

There are thus two aspects under which the nerve centres can be studied. The one is, for the most part, morphological and consists in the following out of the tracts of fibres which connect its various parts together. The other consists in the investigation of the means by which functional connection is established for the performance of different co-ordinated actions.

There is reason to suppose that distinct effectors, as muscle cells, made their appearance in the course of evolution previously to nervous tissue. The receptor, in order to increase the sensitiveness to outer agencies, appears next in close connection with the effector and, as it becomes necessary for effectors at greater and greater distances from the receptor to be acted on, this receptor cell is prolonged in the form of a nerve fibre. Later, an adjuster cell is formed between the receptor and effector, giving the opportunity of connecting up various receptors and effectors together.

The constituent cells of the lowest central nervous systems, as that of the jelly-fish, seem to be in direct protoplasmic continuity with one another, forming a true network. But, very early in evolution, we find that this mode of connection ceases and the cells, now known as "*neurones*," although in functional continuity, are separated from each other where contact takes place, the "*synapse*," by a *membrane*, which plays a very important part in the mechanism of the reactions which take place in nerve centres.

The simplest of these mechanisms is that in which two neurones only are concerned: the receptor neurone, whose cell body is outside the nerve centre, and the motor neurone, whose cell body is within the nervous centre, but whose long nerve fibre, or *axon*, passes out to some peripheral effector organ, such as a muscle. This is a *reflex arc*, in which a sensory impression gives rise to a motor response. It is the functional unit of the nervous system, as the neurone is its anatomical one.

Even as low as the earthworm, a new set of neurones is to be found, *association* neurones, which lie entirely within the central nervous system. These serve to connect the neurones of one segment with those of other segments; although, in this case, they rarely extend beyond two segments.

The progress of the nervous centres in complexity and efficiency of integration depends essentially on the formation of longer and longer association neurones. These form, as it were, loops, consisting often of several neurones, which extend further and further from the original simple arc of two neurones, so that the most highly developed parts of the system, such as the cerebral cortex of the higher vertebrates, consist of association neurones only.

The neurone itself, as a cell, possesses the general properties of protoplasm. The cell body, containing the nucleus, consists of a viscous fluid, with numerous very fine granules in suspension. In life, there is no evidence of the presence of Nissl bodies or neuro-fibrils, and there is every reason to suppose that they are artefacts, as we see them in fixed preparations, although the substance out of which they are formed must have been present in the living cells.

The protoplasm itself does not seem to be essential for conduction of impulses

serving for reflexes, but probably acts as a means of reinforcing the strength of disturbances and certainly, with its nucleus, acts as the nutritive centre of the neurone.

Owing to the protoplasmic nature of the cell bodies, nerve centres are very sensitive to deprivation of oxygen. No metabolic process other than oxidation, with evolution of carbon dioxide, has been shown to be present in the normal activity of nerve centres.

A true nerve network, with central functions, such as that of reflex action, does not appear to exist outside of the very simplest types of nervous system. The plexuses of distribution of the nerves to smooth muscle and related structures serve only for conduction, not for initiation of impulses.

The properties of the *synaptic membrane* are of great importance. Such properties of membranes as those described in the earlier chapters of this book must be shown by it. The phenomena of fatigue, summation, irreciprocal conduction, excitation and inhibition are connected with this membrane.

The use of one motor neurone by several receptors, brought about by the existence of these modifiable synaptic membranes, enables great economy and integrative efficiency to be obtained. This is the principle of the "*final common path*" of Sherrington.

Although, in rare instances, a reflex arc may consist of two neurones only, receptor and motor, in the great majority of cases at least three are present, the additional one being a longer or shorter association neurone. The whole of the arc, with the exception of the motor neurone (final common path), may, for convenience, be called the *afferent arc*.

The cerebral ganglion, or brain, is developed in the anterior end, or head, of an animal in connection with the formation of the elaborate system of the "distance receptors," which enable the organism to take account of occurrences not in immediate contact with it.

The reactions of the highest part, the *cortex cerebri*, show, in contradistinction to the reflexes of the spinal cord, much greater possibilities of modification by events elsewhere and by previous activity. Inhibitory phenomena are especially noticeable and the power of changing excitation into inhibition, and vice versa, is a characteristic function of the cortex. Other properties are discussed in the text.

The sympathetic system, as well as the autonomic or visceral system in general, is not an independent nervous system, but an outflow or outflows of efferent fibres from particular regions of the central nervous system. Its characteristic is the presence of synapses with secondary neurones, either in the sympathetic chain, or more peripherally; the axons of these second neurones are non-medullated. The visceral system develops by outgrowths of chains of *cells*, whereas the somatic system is formed by outgrowth of *axons* from cells in the centres.

LITERATURE

General.

Bethe (1903). Sherrington (1906). Starling (1920). Washburn (1909).

Origin.

Parker (1909, 1911, 1918).

Properties of the Neurone.

Ross Harrison (1910). Mott (1912).

Nerve Networks.

Hofmann (1907).

Cerebral Cortex.

Graham Brown and Sherrington (1912). Mott (1910). Graham Brown (1916). Head (1918).

Speech.

Mott (1910).

Sympathetic and Visceral System.

Gaskell (1916). Langley (1900).

CHAPTER XVI

REFLEX ACTION

As pointed out in the preceding chapter, the reflex is to be regarded as the functional unit of the nervous mechanism. Its general nature was described in that place. In exceptional cases, such as the knee-jerk, the reflex arc may, apparently, consist of two neurones only, but, as a general rule, three at least are contained in it, as in the scratch reflex.

SPINAL REFLEXES

For the investigation of the characteristic properties of the spinal reflexes, it is clearly necessary to obtain a preparation in which the spinal cord is separated from the higher centres and has recovered from shock. Such an animal is called by Sherrington the spinal animal, and it is by his work that the possibility of maintaining such animals alive and healthy has been demonstrated. Nearly all the results to be described below are due to Sherrington, whose portrait will be found in Fig. 150.

Sherrington then sums up the chief differences between conduction in nerve trunks and in reflex arcs as follows (1906, p. 14): "Conduction in reflex arcs exhibits (1) slower speed as measured by the latent period between application of stimulus and appearance of end-effect, this difference being greater for weak stimuli than for strong; (2) less close correspondence between the moment of cessation of stimulus and the moment of cessation of end-effect, *i.e.*, there is a marked 'after-discharge'; (3) less close correspondence between rhythm of stimulus and rhythm of end-effect; (4) less close correspondence between the grading of intensity of the stimulus and the grading of intensity of the end-effect; (5) considerable resistance to passage of a single nerve impulse, but a resistance easily forced by a succession of impulses (temporal summation); (6) irreversibility of direction instead of reversibility as in nerve trunks; (7) fatigability in contrast with the comparative unfatigability of nerve trunks; (8) much greater variability of the threshold value of stimulus than in nerve trunk; (9) refractory period, 'bahnung' (or facilitation), inhibition and shock, in degrees unknown for nerve trunks; (10) much greater dependence on blood-circulation, oxygen (Verworn, Winterstein, von Baeyer, etc.); (11) much greater susceptibility to various drugs, anæsthetics."

These differences are obviously due to the passage through synaptic junctions; perhaps, in some cases, passage through the cytoplasm of the cell body of some constituent neurone may play a part.

We will consider some of these in a little more detail.

Latent Period.—The measurements of Jolly (1910) in the case of the flexion reflex and the knee-jerk have been given on page 476 above.

The more intense the stimulus, the shorter the latent period, so that with intense stimuli to the afferent nerve, the delay may scarcely exceed that of the conduction along the nerve trunks alone. The difference between strong and weak stimulation may amount to as much as ten times or more. This delay might be due to the time occupied in setting the synapse into a state of capability of transmission, but experiments by Sherrington (1906, p. 24) did not support this view. A reflex was evoked by a weak stimulus, with a certain latency; the strength of the stimulus was then suddenly increased, and the latent

period compared with that of the reflex evoked by the same strong stimulus when applied as an initial one. In the first case, the synapse had been already prepared, in the latter case, not. The latent period was, indeed, a little shorter in the former case, but not sufficiently so to give any support to the view mentioned.

The delay must, therefore, be chiefly in the actual transmission, and not in making the synapse conductive, nor in an amœboid movement of protoplasmic processes into contact with each other.

Incidentally, the fact shows the importance of the cutting off by inhibition of impulses which are not wanted, since the channels to the final common path on the efferent side seem to be always open; no preparation is needed.

The latent time of a reflex inhibition is no longer than that of an excitation.

After-Discharge.—The discharge from a reflex arc does not, as a rule, cease when the stimulus ceases, often lasting for five seconds or more. The duration is proportional to the intensity of the stimulus. With a weak stimulus, the response may not appear at all until after the actual period of stimulation has passed.

The after-discharge can be cut short sharply by an inhibitory influence, as shown in Fig. 107 (page 388). This rapid arrest is an arrangement for successive interchange of reflexes. It prepares the neurone for another stimulation.

Summation.—Although, as we have seen, there is a phenomenon of this kind to be met with in the case of nerve itself, it is of a limited kind compared with that shown by reflexes. A scratch reflex cannot be elicited by a single induction shock, however strong. On the other hand, very feeble shocks, if they follow one another at not too short intervals, sooner or later evoke a response. In one case, no effect was produced until after forty-four shocks at eighteen per second had been applied to the skin.

Sincerely yours
C. S. Sherrington.

FIG. 150. PHOTOGRAPH OF C. S. SHERRINGTON.
(From a negative in the possession of Mrs Sherrington.)

The flexion reflex, that is flexion of the knee produced by nocuous or electrical stimulation of the skin of the foot, differs from nearly all other reflexes by being elicitable by a single shock.

Facilitation.—This word is used by Sherrington as a translation of "Bahnung." One aspect of the phenomenon is the summation mentioned in the preceding

paragraph. It is also to be seen in the fact that when a reflex produced from two different receptors employs the same final common path, simultaneous application of stimuli to both receptors evokes a larger response than if applied to either alone. This may also be called "reinforcement," and plays an important part in the behaviour of an organism to the various stimuli playing upon it at one time. These combinations of stimuli form, as Sherrington puts it, "constellations of stimuli." It must also be remembered that a reflex not only takes possession of

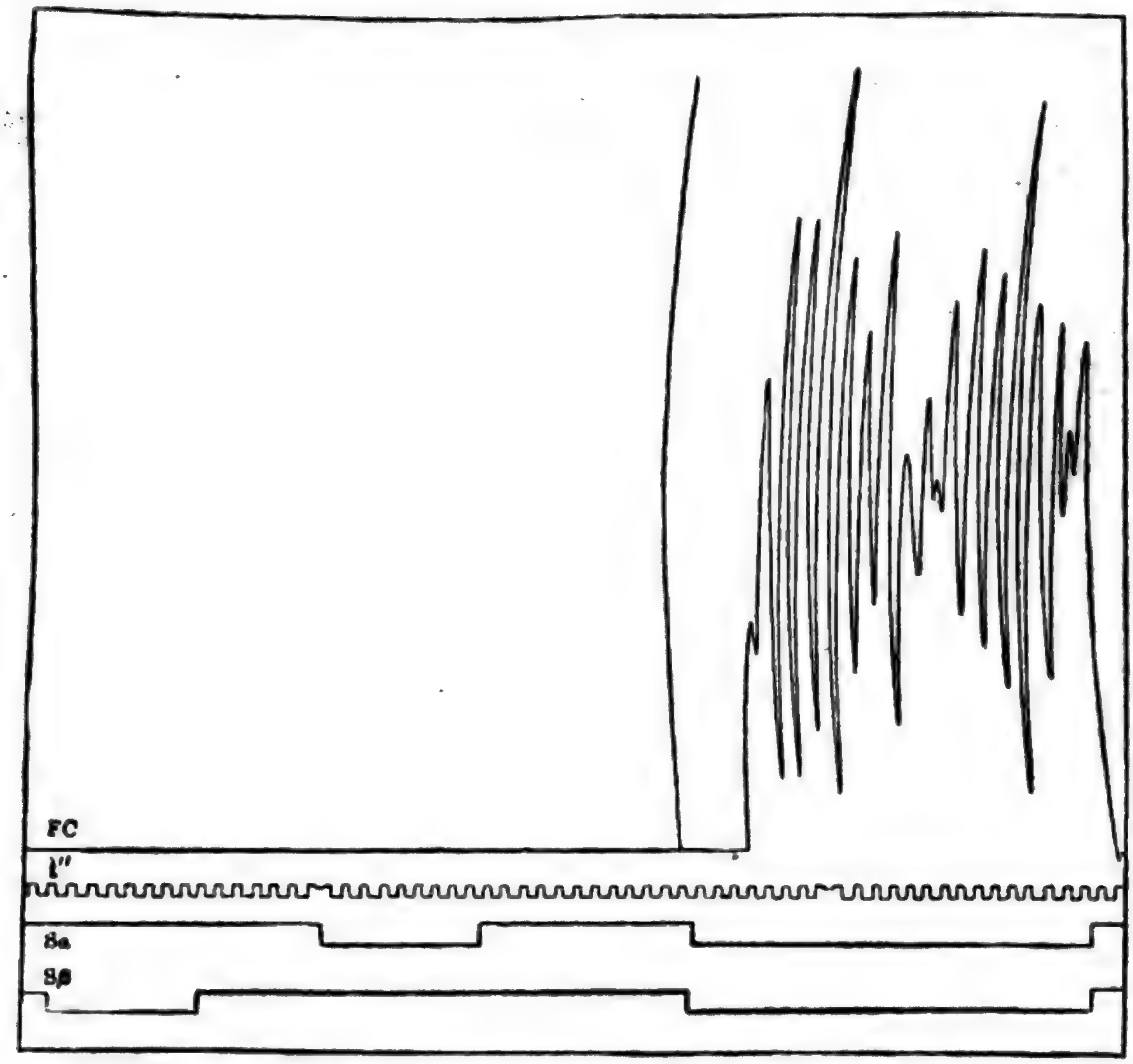

FIG. 151. SUMMATION EFFECT (IMMEDIATE SPINAL INDUCTION) BETWEEN THE ARCS $R\alpha$ AND $R\beta$ OF THE DIAGRAM GIVEN IN FIG. 146 (page 476).

FC, Myogram of the flexor muscle of the hip.

Sα, Signal marking period of stimulation of the skin belonging to arc *Rα* of the shoulder skin. The strength of the stimulus is subminimal, so that there is no reflex response.

Sβ, Signal marking stimulation, also subminimal, of a point of the shoulder skin 8 cm. from *Rα*.

Though the two stimuli applied separately are each unable to evoke the reflex, when applied contemporaneously they quickly evoke the reflex.

The two arcs *Rα* and *Rβ*, therefore, reinforce each other in their action on the final common path, *FC*. Time in fifths of seconds. Read from left to right.

(Sherrington, 1906, pp. 119 and 121.)

certain final common paths, but also of those whose muscles would oppose those of the reflex itself. It inhibits the opposing neurones from being set into action by other reflexes at the same time.

Induction.—Closely allied to the preceding are the phenomena of induction. If the receptive skin area for the scratch reflex is stimulated at one point with subminimal intensity, a reaction may be evoked if another point in the same area be stimulated at the same time, also with subminimal intensity. Such reinforcement is called by Sherrington (1906, p. 120), *immediate spinal induction* (see Fig. 151). It appears that both stimuli act on the same set of neurones composing

the final common path, and, indeed, on the *whole* of the motor neurones of which it consists. This is shown by the fact that two weak stimuli may be made to alternate with each other, each stimulus of the one coming between those of the other. If they acted on different neurones, signs of two rhythms should appear in the muscles of the leg. There is not even a break or interference in the rhythm of the first reflex when the second is added, although the amplitude may be increased. The result must be due to the refractory state induced by each of the first series of stimuli, and in the same neurones (see Fig. 152).

This immediate induction only occurs between allied reflexes, which will be referred to again later.

Successive Induction, on the other hand, is of a different nature. Suppose that we excite an extension reflex in one leg by appropriate stimulation of the opposite leg and with such a strength of stimulus that the reflex is a small one. This is done at regular intervals and the reflex is found to be very constant. In one of the intervals, a strong and prolonged flexion reflex is excited from the limb itself. After this, the extension reflexes are increased both in height and in duration, the effect gradually passing off (Fig. 153). During the flexion reflex, the extension arcs were inhibited and the phenomenon is the same as the "rebound," referred to under the head of inhibition above (page 422). This exaltation, after a period of inhibition, is not shown by merely removing the exciting stimulus, so that it is not due to rest.

It will be clear that the increased excitability produced by one reflex for its antagonist reflex plays a part in the mechanism of such alternating movements as those of stepping or locomotion.

Irreversibility of Direction.—It is a well-established fact that nerve fibres conduct in both directions. The experiments of Bell and Majendie showed, however, that stimulation of the spinal end of a motor root gives no sign of reflex nor of sensation. Similar results of Gotch and Horsley have been mentioned above (page 475).

The experiments of Vészi (1909) showed that fatigue of the reflex mechanism could not be obtained by stimulation of the axons of the motor neurones, and those of Fröhlich (1909, 2) showed that the same thing applies to the simple reflex arc of the stellar ganglion of the Cephalopod. While fatigue of this ganglion can readily be produced by stimulation of the nerves proceeding to it from the cerebral mass, it cannot be fatigued by stimulation of the motor nerves which it gives off to the muscles of the mantle.

We have seen when discussing the properties of the true nerve network of Medusæ that excitation passes in all directions. It appears from fixed preparations that the "neuro-fibrils" are, in this case, continuous from one cell to another, and, although these are probably artefacts, the fact of their continuity suggests

FIG. 152. ABSENCE OF CHANGE OF RHYTHM WHEN PLACE AND RATE OF STIMULATION ARE CHANGED.

Scratch reflex elicited from two skin points, *A* and *B*. The point *A* gave the "low" form of reflex; *B* the "high" form.

The interval between the stimuli at *B* much shorter than at *A*. The slow rate at *A* is marked between the signal lines.

Time in fifths of seconds above.

Note the absence of any sign of interference of rhythm when the second series of stimuli comes in and when the first is cut off. Hence *all* the neurones of the final common path must have been active in *both* cases.

(Sherrington, 1906, 1, p. 12.)

that the substance from which they arise is continuous and not broken by a synapse, as in the higher forms of "polarised" nerve centres.

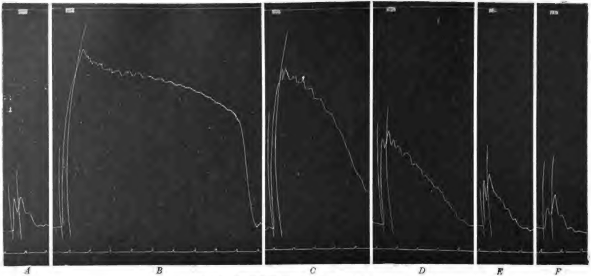

FIG. 153. SUCCESSIVE INDUCTION IN SPINAL REFLEX.

The tracing is that of extension of the knee, evoked at one-minute intervals by stimulation with ten shocks of the skin of a digit of the contralateral limb, of equal intensity in all six cases. The reflex was of small magnitude but of great regularity. Between *A* and *B* a strong flexion reflex of the same limb was provoked, and maintained for thirty-five seconds, by stimulation of the hamstring nerve. This is not given on the tracing, but was, of course, associated with inhibition of the extensors. The extension reflex next following, *B*, although evoked with the same strength of stimulation as *A*, shows great increase in amplitude and duration, especially in after-discharge. The effect subsides gradually, being still obvious at *E*, four minutes after the flexion reflex, but has disappeared at *F* in the fifth minute.

Signal of stimuli at top. Time in seconds below.

(Sherrington, *Proc. Roy. Soc.*, 77**B**, p. 488.)

It would be prèmature to attempt an explanation of why the synaptic membrane is permeable to excitation in one direction only. It may be that it is permeable to one ion only of an electrolytically dissociated colloid, in the way described on page 141 for the system of Congo-red and parchment paper. In such cases, an electrical current can only pass in one direction. But further knowledge is needed.

Refractory Phase.—This characteristic property of muscle and of nerve trunks has been described above. In many reflexes, such as the scratch reflex, it is

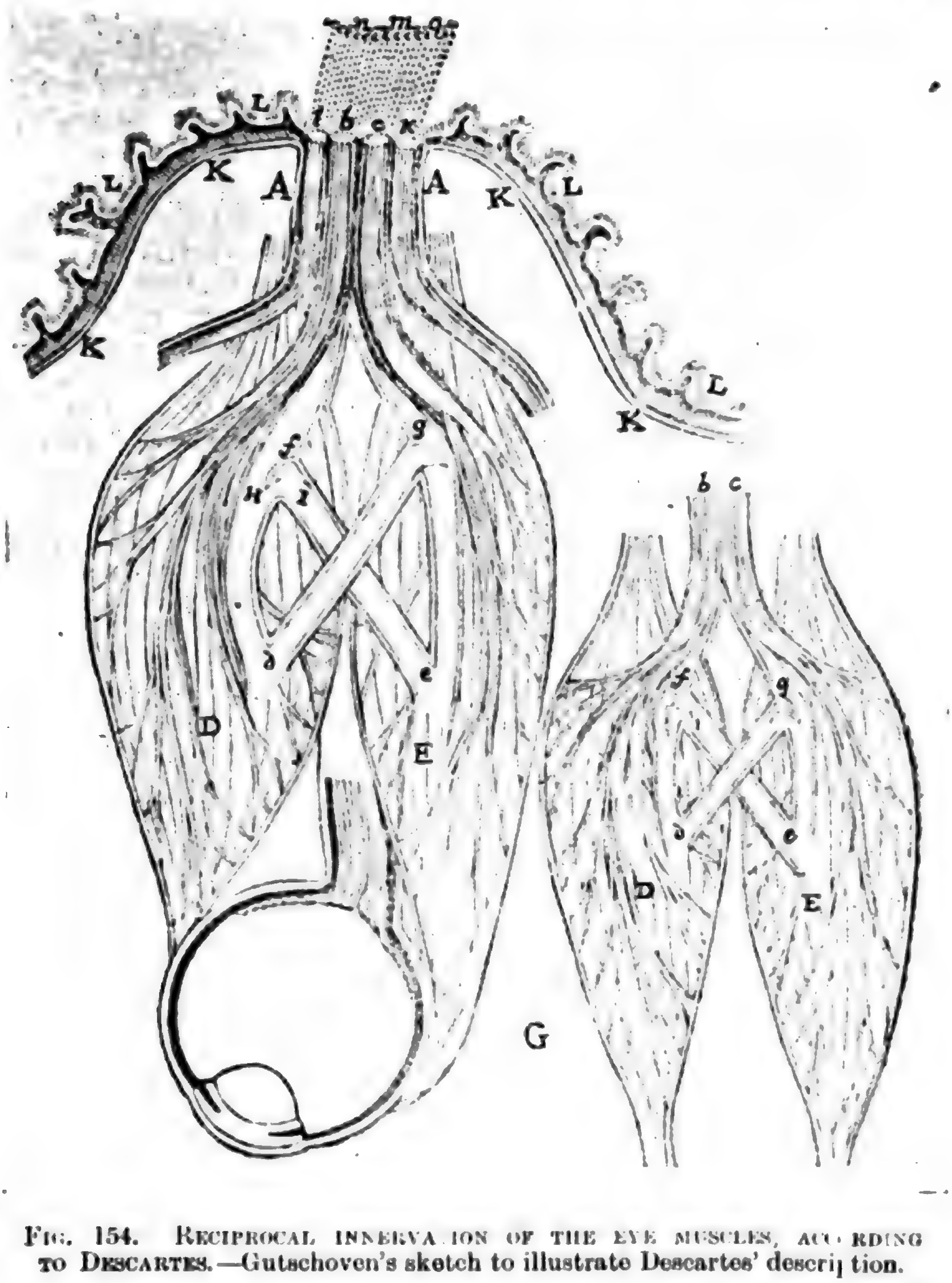

FIG. 154. RECIPROCAL INNERVATION OF THE EYE MUSCLES, ACCORDING TO DESCARTES.—Gutschoven's sketch to illustrate Descartes' description.

(Descartes, 1677, p. 15.)

very marked. This reflex consists of alternate flexion and extension at a rate of about four times per second. This rate is independent of the frequency of the stimulation, so that it is the same when the stimulus is a constant current. High-frequency currents are also very effective (Sherrington, 1906, pp. 48 and 49). Alteration in strength of stimulus has no effect on the rate of discharge. Suppose that the stimuli are applied at the rate of one hundred shocks per second, it is clear that the greater number of these must be ineffective; in other words, they fall in a refractory period.

We have already seen that the reflex arc in this case consists of at least three

neurones, in addition to the muscle fibre at one end and the receptor organs in the skin at the other end. Where in this series are we to place the seat of the refractory period? Now, when the motor neurones, made use of by this reflex, are excited for a different reflex from receptors in the leg itself, this refractory phase does not show itself; the flexion reflex is a steady one. We can exclude, therefore, the motor neurones of the final common path, as well as the muscles themselves. We have seen above that there must be some mechanism common to impulses started at two different spots in the receptive area, even when they are 10 cm. apart. There is, further, no evidence that there is any direct connection between receptor neurones themselves; there is only that due to their synapses with *other neurones* common to both receptors. The conclusion is that the refractory period must be in some neurones on the afferent side of the motor neurones of the final common path, a conclusion which, indeed, seems to be necessary for the proper working of the reflex mechanisms; since the rhythmic movement of scratching would not do for the other reflexes in which the same motor neurones take part. It is interesting to note that the rate of the rhythm is almost identical with that observed by Gotch and Burch (1896) in the discharge of the electrical cell of Malapterurus.

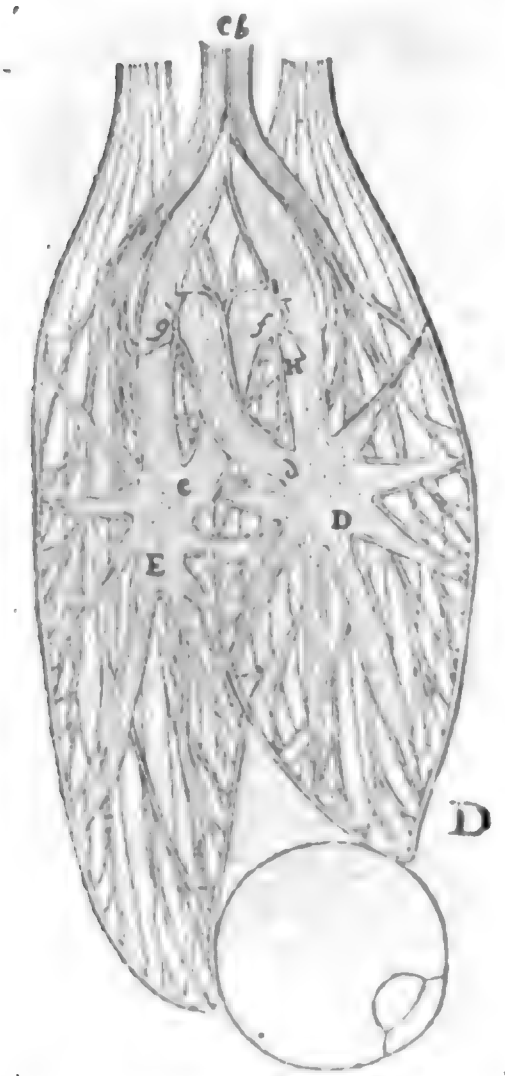

FIG. 155. DESCARTES' ROUGH SKETCH OF THE INNERVATION OF THE EYE MUSCLES.

(Descartes, 1677, p. 16.)

RECIPROCAL INNERVATION

When there are two sets of muscles acting on a movable organ, such as the eye or a part of a limb, in such a way that they antagonise one another, it is clear that, for the effective performance of a particular reflex movement, any contraction of the muscles opposing this movement must be inhibited. Further, the inhibition of the one group must proceed *pari passu* with the excitation of the other group to ensure a well-controlled and steady motion.

This fact was obvious to Descartes (1677), and Figs. 154 and 155 are taken from his treatise "De l'Homme."

The history of this work is of some interest. References are usually made to the Latin translation, "De Homine," but, happening to come into possession of an edition in French brought out by Descartes' friend and disciple, Clerselier, I wondered why the original French manuscript of the author had been translated into Latin and then, apparently, back again into French. On investigation, I found that Descartes, having the fate of Galileo before him, was by no means desirous of offending the ecclesiastical authorities, so that the work remained unpublished at his death in 1650. Clerselier had one copy of the manuscript and another copy was translated into Latin by Schuyl and published at Leyden in 1662. Clerselier, hearing of this, hastened to publish the original French manuscript in Paris. In the second edition, of 1677, he apologises for some errors which, owing to the hurried publication of the first edition, had crept in. So that his second edition appears to be the most accurate representation of the original.

Descartes left some extremely rough sketches, one of which, as copied by Clerselier, "with his best ability," as he says, is given in Fig. 155. The figures with the letter G at the bottom, reproduced in Fig. 154, are by a M. Gutschoven of Louvain. Clerselier made the acquaintance of this gentleman and, finding him to be thoroughly familiar with the views of Descartes from his conversations with the philosopher, commissioned him to draw more figures in order that the text should be more easily understood. A further series of drawings were obtained from a M. de la Forge, whose notes were also added to the book. I have not thought it necessary to reproduce M. de la Forge's figure.

The description of the figures is sufficiently interesting to give it in Descartes' own words and I think that it must be admitted that Gutschoven's diagram makes them more intelligible:

"Voyez aprés cela comment le tuyau, ou petit nerf, bf, se va rendre dans le muscle D, que je suppose estre l'un de ceux qui meuvent l'oeil; et comment y estant il se divise en plusieurs branches, composées d'une peau lâche, qui se peut étendre, ou élargir et retrecir, selon la quantité des Esprits Animaux qui y entrent, ou qui en sortent, et dont les rameaux ou les fibres sont tellement disposées, que lors que les Esprits Animaux entrent dedans, ils font que tout le corps du muscle s'enfle et s'accourcit, et ainsi qu'il tire l'oeil auquel il est attaché; comme au contraire lors qu'ils en ressortent ce muscle se desenfle et se rallonge.

"De plus, voyez qu'outre le tuyau b f, il y en a encore un autre, à sçavoir e f, par où les Esprits Animaux peuvent entrer dans le muscle D, et un autre, à sçavoir d g, par où ils en peuvent sortir. Et que tout de mesme le muscle E, que je suppose servir à mouvoir l'oeil tout au contraire du precedent, reçoit les Esprits Animaux du cerveau par le tuyau c g, et du muscle D par d g, et les renvoye vers D par e f. Et pensez qu'encore qu'il n'y ait aucun passage evident, par où les esprits contenus dans les deux muscles D et E, en puissent sortir, si ce n'est pour entrer de l'un dans l'autre; toutesfois, parce que leurs parties sont fort petites, et mesme qu'elles se subtilisent sans cesse de plus en plus par la force de leur agitation, il s'en échappe tousiours quelques-unes au travers les peaux et des chairs de ces muscles, mais qu'en revanche il y en revient tousiours aussi quelques autres par les deux tuyaux b f, c g.

"Enfin voyez qu'entre les deux tuyaux b f, e f, il y a une certaine petite peau H f i, qui separe ces deux tuyaux, et qui leur sert comme de porte, laquelle a deux replis H et i, tellement disposez, que lors que les Esprits Animaux qui tendent à descendre de b vers H, ont plus de force que ceux qui tendent à monter d'e vers i, ils abbaissent et ouvrent cette peau, donnant ainsi moyen à ceux qui sont dans le muscle E, de couler tres promptement avec eux vers D. Mais lors que ceux qui tendent à monter d'e vers i sont plus forts, ou seulement lors qu'ils sont aussi forts que les autres, ils haussent et ferment cette peau H f i, et ainsi s'empechent eux-mesmes de sortir hors du muscle E; au lieu que s'ils n'ont pas de part et d'autre assez de force pour la pousser, elle demeure naturellement entr' ouverte. Et enfin que si quelques fois les esprits contenus dans le muscle D, tendent à en sortir par b f e, ou d f b, le reply H se peut étendre, et leur en boucher le passage. Et que tout de mesme entre les deux tuyaux c g, d g, il y a une petite peau ou valvule g, semblable à la précedente, qui demeure naturellement entr' ouverte, et qui peut estre fermée par les esprits qui viennent du tuyau d g, et ouverte par ceux qui viennent de c g.

"En suite dequoy il est aisé à entendre que si les Esprits Animaux qui sont dans le cerveau ne tendent point, ou presque point, a couler par les tuyaux b f, c g, les deux petites peaux ou valvules f et g demeurent entr' ouvertes, et ainsi que les deux muscles D et E, sont lâches et sans action; dautant que les Esprits Animaux qu'ils contiennent, passent librement de l'un dans l'autre, prenant leur cours d'e par f, vers d, et reciproquement de d par g vers e. Mais si les esprits qui sont dans le cerveau tendent à entrer avec quelque force dans les deux tuyaux b f, c g, et que cette force soit égale des deux costez, ils ferment aussi-tost les deux passages g et f, et enflent les deux muscles D et E autant qu'ils peuvent, leur faisant par ce moyen tenir et arrester l'oeil ferme en la situation qu'ils le trouvent.

"Puis si ces Esprits qui viennent du cerveau tendent à couler avec plus de force par b f que par c g, ils ferment le petite peau g, et ouvrent f, et ce plus ou moins, selon qu'ils agissent plus ou moins fort; au moyen de quoy les Esprits contenus dans le muscle E se vont rendre dans le muscle D, par le canal e f; et ce plus ou moins viste, selon que la peau f est plus ou moins ouverte: Si bien que le muscle D, d'où ces esprits ne peuvent sortir, s'accourcit, et E se rallonge; et ainsi l'oeil est tourné vers D. Comme au contraire, si les esprits qui sont dans le cerveau tendent à couler avec plus de force par c g que par b f, ils ferment la petite peau f, et ouvrent g; en sorte que les esprits du muscle D retournent aussi tost par le canal d g dans le muscle E, qui par ce moyen s'accourcit, et retire l'oeil de son costé.

"Car vous sçavez bien que ces Esprits, estant comme un vent ou une flamme tres subtile, ne peuvent manquer de couler tres promptement d'un muscle dans l'autre, si tost qu'ils y trouvent quelque passage; encore qu'il n'y ait aucune autre puissance qui les y porte, que la seule inclination qu'ils ont à continuer leur mouvement, suivant les loix de la Nature. Et vous sçavez outre cela, qu'encore qu'ils soient fort mobiles et subtils, ils ne laissent pas d'avoir la force d'enfler et de roidir les muscles où ils sont enfermez; ainsi que l'air qui est dans un balon le durcit, et fait tendre les peaux qui le contiennent." (Clerselier's edition, pp. 15-20.)

It will be remembered how Descartes looked upon the material bodies of man and animals as pure machines, using, in fact, the word itself. In man, this machine is made use of by the soul, which enters into relation with it at the pineal gland. Other animals, which have no souls, are therefore nothing but machines. The cries made by a dog when injured are no more than the noise made by a machine when a part of it breaks off and gets into the wheels. The object of the "Traité de l'Homme" is to show how the working of the human body can be explained on purely mechanical principles. According to Stensen (Steno), who lived from 1631-1686, and whose name is familiar in the denomination of the duct of the parotid gland, Descartes did not pretend to expound the actual structure of man's body, but to describe a machine capable of performing all its functions (quoted by Foster, 1901, p. 62).

It will scarcely escape the notice of the reader how closely the method of description of the

innervation of the eye muscles, as given by Descartes, approaches the "drainage" views of Macdougall and von Uexküll, if we read "neurin," "nerve-energy," "tonus" or "excitation"

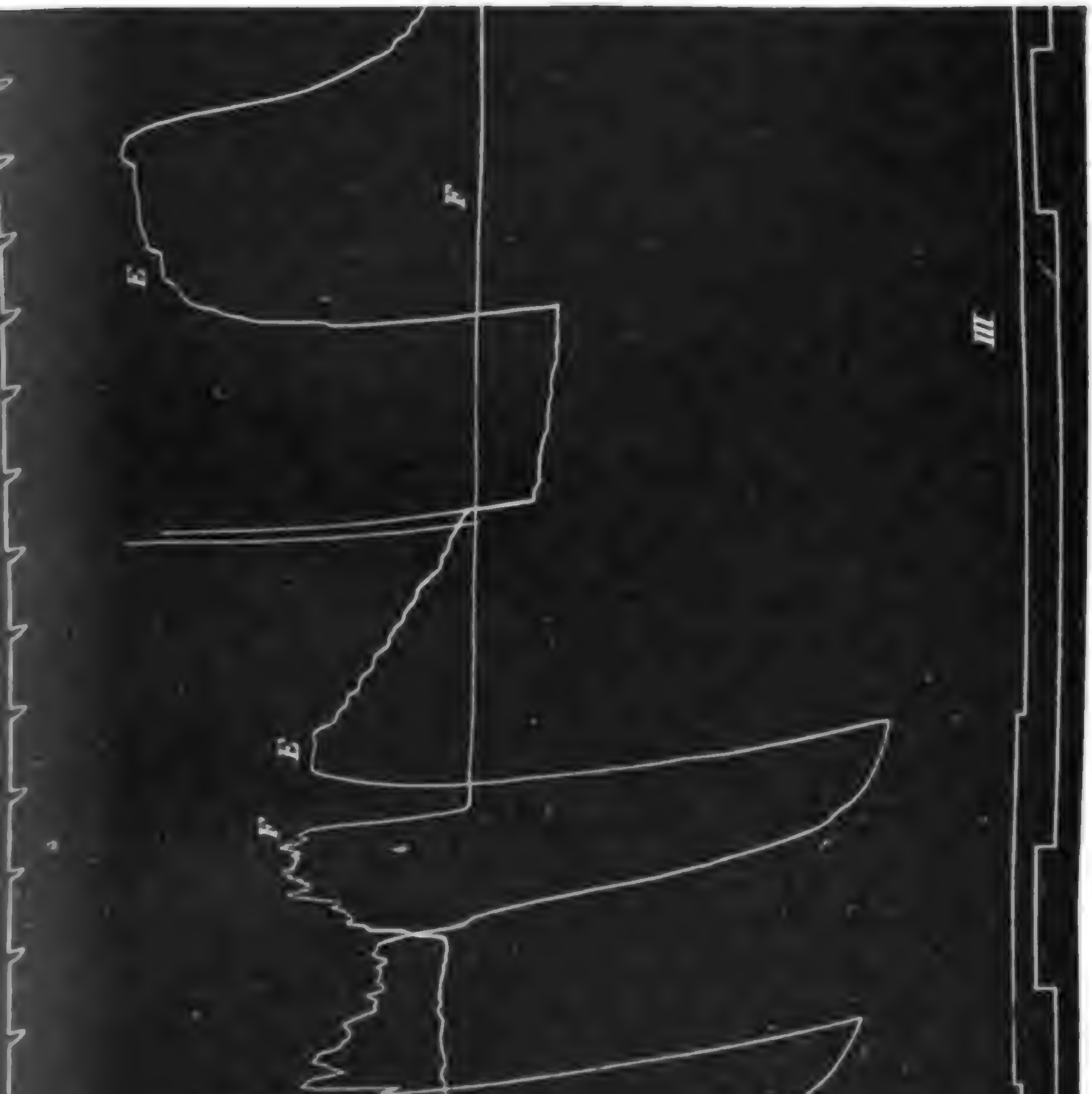

FIG. 156. RECIPROCAL INNERVATION IN REFLEXES TO SKELETAL MUSCLE, TOGETHER WITH ALGEBRAIC SUMMATION OF EXCITATION AND INHIBITION IN THE PROCESS.

E, Tracing of myograph connected to vasto-crurus (extensor). *F*, That of semitendinosus (flexor).
I, Signal for stimulation of ipsilateral afferent nerve (peroneal, which is excitatory for flexors, inhibitory for extensors).
C, Signal for stimulation of contralateral peroneal nerve (inhibitory for flexors, excitatory for extensors).
The control arcs at the beginning show the relative positions of the two myograph levers.
Time in seconds above. Cat, decerebrate preparation.

In *I*, stimulation of the ipsilateral peroneal nerve causes reflex contraction of flexors, inhibition of tonic contraction in extensors.
In *III*, stimulation of contralateral peroneal nerve causes contraction of extensors. Since there was no tonic contraction in the flexors, it is clear that the inhibitory effect on the centre could not show itself. That it was present, however, is shown by the middle part of the tracing *II*. The contraction of the flexor, produced by stimulation of the ipsilateral nerve, is inhibited when stimulation of the contralateral nerve is added (as marked by the lower signal). Note that the balance is nearly complete under these conditions, but that both levers are somewhat on the excitatory side of their resting position, and that there is an indication of rhythmic contractions, especially seen in the extensor muscle.

(Sherrington, 1909, 2, p. [illegible].)

in place of "Esprits Animaux." The essential difference between the present view of reciprocal innervation and that of Descartes is that the latter placed the mechanism in peripheral structures, whereas we know now, *by experiment*, that it is in the nerve centres.

Although this reciprocal relation of antagonistic muscles was present to the minds of various previous physiologists in a certain way, as for example to Meltzer (1883, pp. 215-216), it was not until the work of Sherrington (1892, etc.) that our knowledge of the mechanism became clear and definite.

The phenomenon is shown in a striking way in Fig. 156 (from the paper by Sherrington, 1909, 2, p. 260). The extensor muscle of the knee (vasto-crureus), in a decerebrate cat, is isolated and connected to a tracing lever, similarly the flexor (semi-tendinosus). These muscles are connected to the nerve centres by their nerves, but the connections of all other muscles which might cause movement of the levers are severed. The lower one (C) of the two signal lines at the bottom of the figure indicates, by its rise, stimulation of the central end of an afferent nerve of the leg of the opposite side (contralateral peroneal) and the upper signal (I) marks stimulation of the corresponding nerve of the leg itself under observation (ipselateral). E marks the myograph tracing of the extensor, F that of the flexor. The lever attached to the extensor writes a few millimetres to the right of the lever attached to the flexor. As the preparation was decerebrate, the extensor muscle was in tonic contraction, but not the flexor, since decerebrate tonus affects the muscles of posture only (Sherrington, 1906, p. 302), which counteract gravity. The first stimulation is that of the ipselateral nerve, which produces a flexion reflex. In this reflex we see that, along with the contraction of the flexor muscle, there is a marked inhibition of extensor tone, followed by a rebound (successive spinal induction), as described above. In the *third* stimulation, that of the contralateral nerve, producing extension, inhibition of the flexor cannot show itself on account of the fact that the muscle is already in a state of relaxation, but it can be shown, indirectly, that the centres are inhibited, as by the middle tracing (see description of figure). It is clear that the afferent nerve fibres proceed to the motor neurones of both the antagonist muscles, but, while exciting the one, they inhibit the other. Examination of the figures will show also that, in the rebound contraction, sudden inhibition of the flexor contraction coincides with excitation of the extensor muscle.

Similar phenomena are observed in the movements of the eye brought about by stimulation of the cerebral cortex and in movements of the limbs produced in the same way. Sherrington concludes (1906, p. 285) that the seat of the inhibition in these particular reactions from the cortex is not in the cortex itself, but probably at the ultimate synapse with the final common path, that is, the motor neurone. Certain phenomena to be described later, however, indicate that, although the seat is very near to the final synapse, it is very likely in some intermediate synapse. The phenomena referred to relate to the action of strychnine and of chloroform. It is not to be concluded that, in many other cortical reactions, inhibition of one cortical element is not effected by other cortical elements, in fact, there is every reason to believe that this is the case.

Reciprocal co-ordination was observed by Sherrington (1906, p. 285) in the "willed" movements of the eyeballs in the monkey. The external rectus muscle is supplied by the sixth cranial nerve, so that, if on one side the third and fourth nerves, which supply all the other muscles, are cut, any movements of this eye are due only to changes in the state of contraction of the external rectus. If now an object is moved horizontally in such a way that its movement is followed by means of *contraction* of the external rectus of the normal eye, it is seen that the other eye also follows the movement. Since any movement of this eye must be effected by the external rectus alone, and the movement observed is such as to be brought about by *relaxation*, it follows that the tonus of its centre must be *inhibited* in accurate time and step with excitation of the external rectus of the opposite eye. It is therefore to be presumed that a similar process is going on with regard to the *internal* rectus of the normal eye, which works in conjunction with the external rectus of the other eye.

A kind of reciprocal innervation holds in two cases already dealt with. In the first of these, the "*myenteric reflex*" of the intestine, the mechanism is probably peripheral, although it simulates a reflex. In the second, the opening and closing of the *claw of the crayfish*, the mechanism is not reflex, but of peripheral

nature, as we have seen (page 425). A similar case to the latter is that of the action of the sympathetic nerve on the muscles of the *iris*. Weymouth Reid (1894) showed that, in this dilatation of the pupil, simultaneous contraction of the dilator, radial, muscle and inhibition of tone of the sphincter, circular, muscle takes place.

The *tonic* contraction of muscles concerned in the maintenance of posture has also been shown by Sherrington to be subject to reciprocal innervation.

We shall see later that vasomotor and respiratory reflexes follow the same law.

DOUBLE RECIPROCAL INNERVATION

The simple case of the antagonistic muscles of the knee joint, acted on reflexly by stimulation of one whole afferent nerve, is not like the normal complex state of affairs, although it shows us the elements out of which the latter is constructed. Any particular motor centre is always more or less under a twofold influence of both excitation and of inhibition. This can be studied by taking a pair of antagonistic muscles and *two* afferent nerves, one having the opposite reflex effect to the other, as was done by Sherrington (1909, 2) in the work already referred to. If the two nerves are excited simultaneously, the effect on the movement of the joint depends on the relative strength of the two stimuli. By study of the myograph tracings, such as those of Fig. 156, it is seen that the motor centre of each muscle is under a twofold influence; the discharge of each represents the algebraic sum of the excitatory and inhibitory influences playing upon it. At a particular relative strength, both flexor and extensor centres may discharge, but neither discharge is as great as it would have been if the antagonistic inhibitory influence were absent (see the middle tracing of Fig. 156).

The study of this phenomenon, as Sherrington points out, shows the importance of inhibition, not only as suppressing excitation, but as a delicate adjuster of the intensity of reflex contraction, a method which is probably of frequent occurrence in natural movements.

RHYTHMIC REFLEXES

When the intensities of the two opposing influences on the same centre are nearly equally matched, a rhythmic discharge results. An indication of this is seen in the middle tracing of Fig. 156. If the movements of the right and left legs are observed under these conditions, flexors and extensors are seen to be alternating in contraction on the two sides, so that a stepping movement results; when the one leg is flexed, the other is extended and vice versa. The following explanation is suggested by Sherrington (1913, 2, p. 98): "Reflex inhibition of a centre tends to superinduce in it a state of superactivity, rebound; and conversely, as has long been known, the reflex excitation of a centre tends to superinduce in it a state of depressed activity, fatigue. It is therefore not surprising that when the two antagonistic influences are concurrently at work on a centre, and are nearly balanced, there should result a rhythmic oscillation of the two; and presumably the rate of their alternation will depend largely on the nicety of balance, and on the intensity with which the processes are acting." Forbes (1912, 2, p. 287) points out that if we have two opposing forces, an increasing one (A) acting against a constant one (B), and if B is acting in some way to keep potential energy pent up, then, as soon as A becomes greater than B, the accumulated energy is released and becomes kinetic. The important point in the present connection is that, when once the release of energy has begun, it proceeds until *more* energy is released than is represented by the excess of A over B, owing to the kinetic energy of the current. A tank into which a stream of water is flowing and provided with an outlet at the bottom, closed by a spring, is such a case. Forbes further shows that "biogen" molecules, obeying the law of mass action alone, would give a continuous response to a continuous stimulus. Also that

"to develop rhythm of discharge there must be an approximation to the all-or none law."

Graham Brown (1912, pp. 285-286) states that the phenomena observed by him cannot be accounted for either by a "drainage" theory, or by a metabolic one, nor again by the assumption of a self-generated antagonistic stimulus. He suggests that the respective centres of the corresponding muscles of the two sides act reciprocally *on each other*, the one in excitation inhibiting the other, although, as he admits, the nature of the inhibitory process is not explained thereby.

Further details of this interesting question will be found in the papers by Graham Brown (1912), Forbes (1912, 2) and Sherrington (1913, 1). Fig. 157 illustrates the phenomenon well.

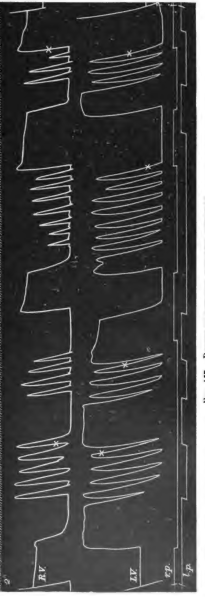

Fig. 157. Rhythmic stepping produced reflexly.

R.V., Myogram of right isolated vasto-crureus muscle. | *r.p.*, Signal for stimulation of right peroneal nerve.
L.V., Myogram of left isolated vasto-crureus muscle. | *l.p.*, Signal for stimulation of left peroneal nerve.
These nerves are antagonistic in their effects on the reflex. Time above in fifths of seconds.

Stimulation of the right nerve causes relaxation of the right muscle, contraction of the left. Stimulation of the left nerve causes contraction of the right muscle, relaxation of the left. When both are stimulated together and the relative strength of the two stimuli is of a certain value, there is an alternation of contraction and relaxation in both muscles, so that contraction of the one coincides with relaxation of the other. In this case the positions of the two secondary coils were, for the right nerve, 15 cm.; for the left nerve, 17 cm.

(Sherrington, 1913, 1, p. 244.)

THE ACTION OF STRYCHNINE AND OF CHLOROFORM

The conversion, by strychnine, of central inhibition into excitation, and that of excitation into inhibition by chloroform, were described above (page 428).

As we shall see in the next chapter, Magnus has shown that the extensor tonus of the muscles of

the limbs is greatly influenced by stimuli from the receptors of the labyrinth and the neck, and that this extensor tonus is associated with inhibition of flexors. Now, in this particular case of reciprocal inhibition, it has been shown by Magnus and Wolf (1913, p. 458), that the inhibitory component cannot be reversed even by so large a dose of strychnine that convulsions made further experiments impossible. These observations were made on triceps brachii and on vasto-crureus isolated. Since it was shown by Sherrington that a small dose of strychnine reverses the inhibitory component of reflexes from afferent nerves of the limb observed, in which these same muscles are employed, it follows that the same muscle in one reflex may respond with reversed inhibition (*i.e.*, excitation), and in another reflex with normal inhibition. Magnus and Wolf rightly draw the conclusion that no "anatomical" scheme of connections can explain this fact. It may be that there are two independent synapses with the final common path, unequally sensitive to strychnine, or, in accordance with the conclusion to which I was led by my observations on vasomotor reflexes, that the drug acts on some intermediate synaptic membranes on the afferent side, synapses which are not part of the path common to the two different reflexes. One of these, that in the reflex arc of the afferent nerve from the limb itself, is more sensitive to the drug than those of the posture reflexes from labyrinth and neck. But it seems that either hypothesis would suffice.

It will be clear, in any case, what havoc strychnine and tetanus toxin must play with reciprocal innervation in the organism. As Sherrington says (1905, p. 296): "The sufferer is subjected to a disorder of co-ordination which, though not necessarily of itself accompanied by physical pain, must inflict on the mind, which still remains clear, a torture inexpressibly distressing. Each attempt to execute certain muscular acts of vital importance, such as the taking of food, is defeated because from the attempt results an act exactly the opposite to that intended. The endeavour to open the jaw to take food or drink induces closure of the jaw, because the normal inhibition of the stronger set of muscles—the closing muscles—is by the agent converted into excitation of them. Moreover, the various reflex arcs that cause inhibition of these muscles not only cause excitation of them instead, but are, periodically or more or less constantly, in a state of hyper-excitement; and yet attempt on the part of the sufferer to restrain, to inhibit, their reflex reaction, instead of relaxing them, only heightens their excitation further, and thus exacerbates a rigidity or a convulsion already in progress." Sherrington thinks it probable that the action of the toxin of rabies lies in a similar effect on the mechanisms regulating swallowing and respiration.

INTERACTION OF REFLEXES

Various reflexes use the same final common path for different purposes or for similar purposes. Afferent arcs which use it for different purposes cannot have possession of it simultaneously and they must take their turns, as it were. One reflex may defer or cut short another. This takes place even if they are both associated with excitation of the motor neurone, if they use the final common path in a different way, as regards time relations, and so on. Such cases are the flexion reflex and the scratch reflex of the leg muscles. These are "antagonistic" reflexes. "Allied" reflexes act together and frequently reinforce one another.

The function of the receptors of the muscle itself, "proprio-ceptors" as we shall learn to call them, is of importance in this process. According to Sherrington (1906, p. 341, and 1909, 3, p. 155), their function is to cut short a reflex and prepare the arc for another one. Thus, a normal muscle, excited to reflex contraction, can be inhibited by stretching it, whereas, in a muscle deprived of its proprio-ceptive afferent fibres by section of the dorsal roots, this cannot be done.

COMPOUND REFLEXES

Certain reflexes may combine together to form a definite co-ordination, which may be either simultaneous or successive. In the latter case, the result

of one reaction excites another, and so on. Thus the reflex protrusion of the frog's tongue, excited by the sight of a fly, provides the stimulus (contact with the mucous membrane of the mouth) which causes closure of the mouth, swallowing of the fly, and so on, in series. In such cases as the scratch reflex, where the conditions can be readily controlled, each reflex increases the excitability of the reflex arc for the next succeeding one. The question of compound reflexes is a large one. Sherrington's book (1906, Chapters IV., V. and VI.) should be consulted.

FATIGUE

Since, as we have seen (page 423), the seat of reflex fatigue is not in the final motor neurone itself, it is clear that the possession of this final common path by a new reflex is considerably affected by the fatigue of a previous reflex. The value of this fatigue of an intermediate synapse is to prevent too long possession of an effector by a particular reflex. Fatigue of a certain reflex enables a second one to obtain possession of the effector, although the stimulus exciting the former reflex may be still going on. The final motor neurone is comparatively incapable of fatigue.

Fatigue of a steady reflex, such as the flexion reflex of the knee, is first shown by its becoming rhythmic.

Although a reflex arc is soon fatigued, it recovers again fairly rapidly; its power of responding again may be very considerable even after ten seconds of rest.

The diminution of its excitability is gradual, so that a weak stimulus ceases to be effective earlier than a strong one does.

A reflex may cease either from fatigue or from inhibition. In a rhythmic reflex, such as the scratch reflex, the difference can be seen. In the former case, the beats become slower, and each beat is more prolonged and sluggish; in the latter case, there is no change in rate nor in the duration of each beat. They are usually abolished altogether by inhibition, without any previous change, although the amplitude may sometimes be reduced.

NOCICEPTIVE REFLEXES

Reflexes that protect an animal from injury are usually prepotent, that is, they displace others. The receptors are probably free nerve endings, since any form of nocuous stimulus is capable of exciting nerve fibres, and there is no need of recognition of the kind of stimulus. Great sensibility to small stimuli, so important in the higher senses, would be a disadvantage in this case. The scratch reflex in the spinal animal can be driven from possession of the final common path by a flexion reflex produced by a pin-prick in the foot. These nociceptive reflexes recover first after spinal transection. Thus, the abovementioned flexion reflex can be obtained earlier than that next described, namely,

THE EXTENSOR THRUST

When the spinal cord has considerably recovered from the shock of section, gentle pressure between the paws will often produce an *extension* reflex, in which the leg is straightened out. This effect is similar to that which is caused by contact with the ground in walking.

Description of the great variety of individual reflexes would be out of place in this book. Those to the viscera, the heart, and the blood vessels are described in other chapters.

AUTOTOMY

We may devote a few words to a peculiar reflex met with in certain crustacea. If a crab be picked up by one of its ambulatory appendages, it generally, by a powerful muscular contraction, breaks this leg off at a particular place and so obtains freedom. This mechanism was first investigated by Fredericq and more recently by Roskam (1913). The second segment of the leg in the crab consists

of two parts, which are distinct members in most crustacea and united by a movable joint. In this animal, however, in place of a joint, there is a double membrane, whose two components are not very firmly united. In the middle of the membrane there is an aperture, through which the nerve and blood vessels pass. Certain muscles are so arranged that, by a powerful contraction, they separate apart the two layers of the membrane. Thus no soft parts are torn, except the nerve and blood vessels; there is practically no bleeding and the peripheral part of the appendage is rapidly regenerated.

METHODS

There are two mammalian preparations which are very useful for the study of reflex action. The "decerebrate," described by Sherrington (1898), for which a cat is best, retains all parts of the central nervous system below the posterior colliculi and shows tonic rigidity of extensor muscles. It is therefore valuable for the investigation of inhibition. The other preparation is decapitated and therefore spinal only (Sherrington, 1909, 1). It is important that the operative procedures in both cases, especially the section of the crura or the spinal cord, should be done under deep anæsthesia; a considerable amount of shock is thus avoided. The vagus nerves should also be divided previously; unless, in the decerebrate preparation, they are required for the purpose of reflexes.

CONDITIONED REFLEXES

Pavlov (1910) states that he was struck by the fact that when the physiologist leaves the study of the simpler parts of the central nervous system, which he has investigated by the observation of reflexes, and proceeds to the higher parts, especially to the cerebral cortex, his methods suddenly change. He gives up observation of the relation between external phenomena and the reaction of the organism to them and introduces psychological ideas, derived from his own internal consciousness.

To extend to the higher centres the method of observing what changes in the organism are correlated with external changes might appear too difficult, but Pavlov has succeeded in doing so to a remarkable degree. The method used is that which he calls "conditioned" reflexes. Unfortunately, up to the present, the experimental results are not easy of access, most of the papers being published in Russian, and it is difficult to follow the train of argument apart from the actual facts.

The complexity turned out to be less than was expected, so far as the response itself was concerned. The production is easy under the right conditions. The complexity depends on the inhibition produced by events taking place independently, all of which exert their influence on the newly-formed reflex and must be duly controlled.

There are two fundamental mechanisms concerned. Firstly, that of *temporary association*, by which external phenomena are brought into connection with reactions of the organism. And secondly, that of *analysers*.

The first, as we have seen in the preceding chapter, is a general property of nerve centres, but becomes more and more complex and *modifiable* in the course of the evolution of the higher centres. Pavlov makes use of it in a definite manner, which I will endeavour to make plain.

In the lower centres, the reflexes are of remarkable regularity, as will have been seen in the previous part of the present chapter. They can, as a rule, be reckoned upon to follow a particular stimulus without fail. They are, in fact, "unconditioned." In the higher centres, the result that follows a particular stimulus depends upon a much greater number of conditions, sometimes no obvious result happens at all. Various "temporary combinations" are formed and we have "conditioned" reflexes. It is unnecessary to remark that the difference is really only one of degree, since no reflex can be said to be absolutely unconditioned.

Now, one of the most essential relationships between the organism and the outer world is that of food. In course of evolution, the means by which the

presence of food becomes known increase in number and complexity with the differentiation of receptors. Thus, sometimes one, sometimes another phenomenon of the outer world becomes a sign of food and the impossibility of other than temporary connections is obvious. It is a case of the telephone exchange again, an illustration used also by Pavlov (1910, p. 9).

How are these temporary combinations made? How is the conditioned reflex formed? It is this: if a new, indifferent, external stimulus is many times present along with one which has already a definite response, the subsequent presentation of the new stimulus alone causes the reflex to be given. The reflex arc has now taken into connection with itself an additional afferent neurone, but not for an indefinite time and unconditionally, as we shall see presently. At the risk of some degree of repetition, it seems advisable to give an illustration. A dog, when given food, secretes saliva, as is well known. Suppose that every time the food is given, a particular bell is rung. After a number of repetitions of the combination of bell and food, the sound of the bell alone is found to cause secretion of saliva. A conditioned reflex to the sound of the bell has been formed. This is a very simple case, but the investigation of the various influences to which it is subject leads to a great deal of valuable information.

The work of Pavlov and his collaborators has, in fact, been hitherto concerned with such a comparatively simple case. The salivary glands have many advantages for the purpose. They can work alone, not being parts of a complicated system. The observation of the effect can be made quantitative, by recording the number of drops of saliva secreted. The operation necessary to make a salivary fistula is very simple and does not interfere with the normal state of the animal.

When food is taken into the mouth, stimulation of the various receptors in the mucous membrane brings about reflex secretion. This is the primitive, unconditioned reflex, present even without the higher parts of the brain. But it can be modified, as every one knows. The sight, or even thought, of food may excite secretion; this is "psychical" secretion and it requires the highest parts of the brain. Fear may prevent the secretion, so that dry food cannot be swallowed.

Now it is just this aspect that can be made into conditioned reflexes. Any phenomenon of the outer world, for which the animal in question possesses appropriate receptors, can be brought into temporary association with salivary secretion, so that it becomes an exciter of secretion, if only it has been frequently presented at the same time with the unconditioned reflex stimulus, food in the mouth.

The study is concerned with the different kinds of stimuli, their mutual effect on one another, and so on. Since a number may be present at one time, there is a great variety of possibilities of *inhibition*, of which Pavlov distinguishes two kinds, external and internal. All kinds of external phenomena may give rise to *external inhibition*. During the course of the formation of a conditioned reflex, especially in the early stages, a very slight outside disturbance may prevent its proper production. Thus, Dr Anrep informs me that he was engaged in the production of a conditioned reflex to a particular metronome beat. It was in the winter time and, just as he presented the food, the laboratory servant began to scrape away the snow at the entrance of the building. The effect of the intended stimulus was at once done away with; the dog's attention was diverted, as the psychologist would put it, and the experiment spoiled for a time. As regards internal inhibition, it is found that a conditioned reflex, say salivary secretion on the sound of a bell, if repeated several times without the subsequent presentation of food, loses its effect, its proper consummation not being arrived at. This is merely temporary internal inhibition, since the reflex returns of itself after a rest.

The "*analysers*" are what have been called sense organs, or mechanisms of sensation, whose function it is to separate and distinguish the complicated phenomena of the outer world. Many of the facts already worked out by physiologists belong to Pavlov's category of conditioned reflexes. When, for instance, a certain combination of stimuli, arising from the retina and from the eye muscles, has several times been found to coincide with the touch stimuli of an object of a given size, the combination becomes the conditioned stimulus of the actual size of

the object. As we shall see in more detail in the following chapter, the analyser consists of something more complex than the peripheral receptors alone. It includes these, but is continued to their central connections in the brain, and these latter are often very complex, reaching to the highest centres. As an

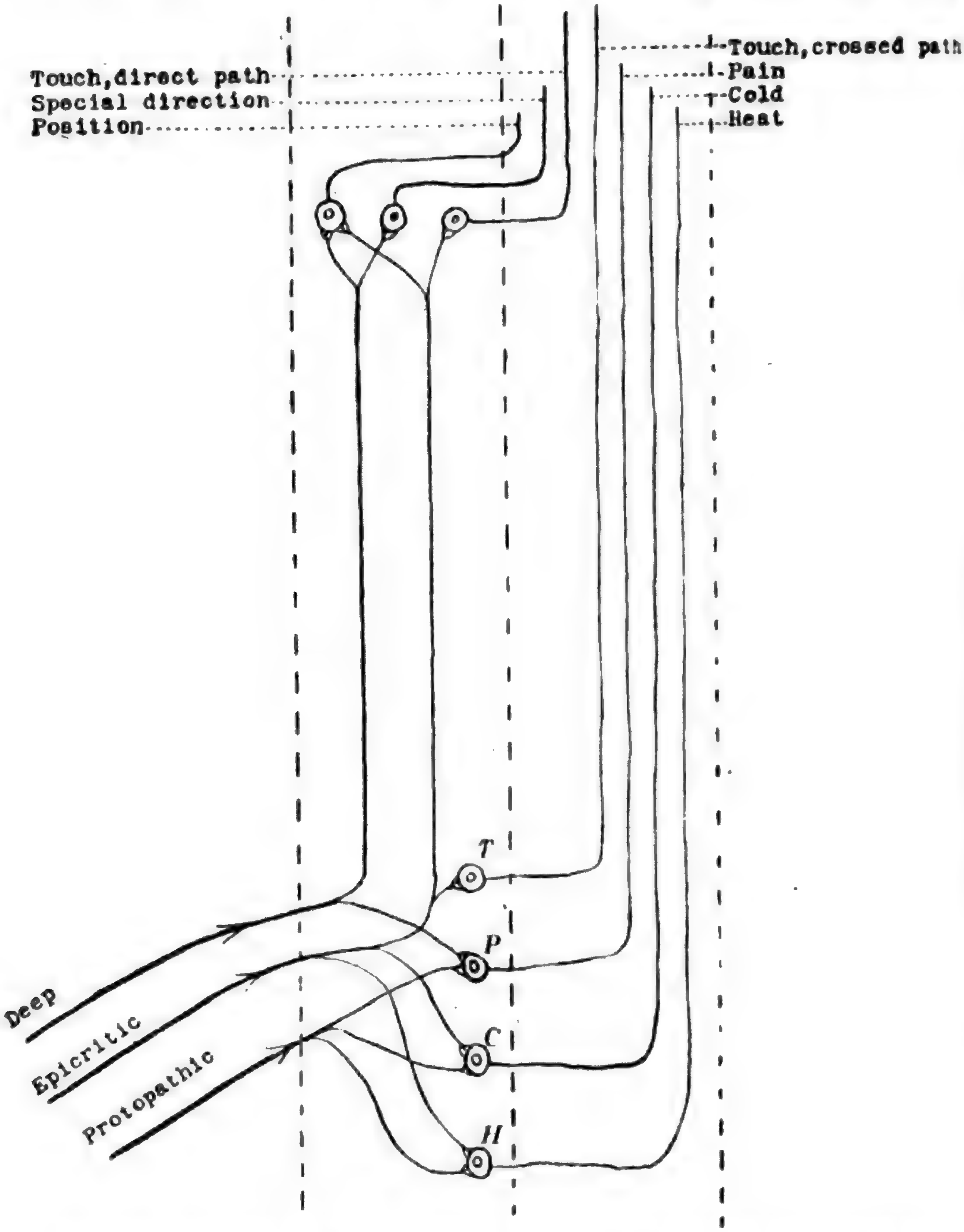

FIG. 158. DIAGRAM OF FIRST STAGES OF CENTRAL ANALYSIS AND INTEGRATION OF SENSORY IMPULSES FROM THE SKIN.

The outer dotted lines mark the lateral boundaries of the spinal cord. The centre line is the middle of the cord. The letters, *T*, *P*, *C*, *H*, refer to synapses with cells concerned with touch, pain, cold, and heat respectively. The lines represent bundles of nerve fibres.

(Head.)

example, Fig. 158, which is a diagram given by Head of the partial analysis and reintegration, in the lower centres of the spinal cord and the bulb, of the three different sets of receptors in the skin and the underlying structures of the hand, may be glanced at. The meaning of the names "protopathic," "epicritic," and "deep" sensibility will be explained later (page 514). For the present, we may

take the first as being associated with pain, the second with the fine, discriminating, higher receptors of touch, heat, and cold, and the last as the Pacinian and similar receptors, connected with the muscular sense and pressure.

It will be clear that the method of conditioned reflexes presents great opportunities of testing the delicacy of the appreciation of external forces. The dog has been found, in this way, to be able to distinguish small differences of the pitch of musical notes.

For example, a note of 100 vibrations per second has been made into a conditioned stimulus by presentation along with food, so that on hearing this note by itself, secretion results. But no effect is produced by a note of 104 vibrations, nor by one of 96. A similar conditioned reflex to electrical stimulation of a spot on the skin ceases to appear when the electrodes are moved 1 cm. away.

This differentiation is found to be brought about by inhibition, that is, by exclusion of all parts of the analyser with the exception of a limited region.

The co-operation of inhibition in the formation of conditioned reflexes may be seen thus: an electrical current sufficiently strong to give signs of pain when applied to the skin, is made the signal of a conditioned reflex when applied to a particular spot. It is found now that it gives signs of pain no longer, but, if moved 1 cm. away, there is no secretion but there are signs of pain. In practice, of course, it is necessary to shave the spot and mark it for future accurate localisation.

We may here pause for a moment to note the difference between the spinal and the higher centres in regard to nociceptive reflexes. We saw that these reflexes are prepotent in the former case, but the conditioned reflex in the case quoted above has obtained the mastery over one. Nocuous skin stimuli can even be made into a conditioned stimulus for the feeding reflex, but not when applied over bone, nor when acid in the mouth is the stimulus for the unconditioned reflex instead of food. So that nocuous stimuli are more difficult to deal with, even by the higher centres, and the mastery over them is only a qualified one.

A spot which has been made the signal for a conditioned reflex, by presentation of food along with the stimulation of the spot, is called an "*active*" spot. It is necessary to distinguish between an "*inactive*" and an "*indifferent*" spot. The latter name is given to spots which have not been used as signals for any particular purpose; while an "inactive" spot is one which has been made, by stimulation, the signal for non-presentation of food; that is, as it were, a reflex for "no food," and is associated with *absence* of salivary secretion.

The following experiment presents several points of interest (Pavlov, 1912, pp. 330-331). Along a series of spots on the hind leg, there were arranged five devices for producing mechanical stimulation of the skin. The stimulus was equal in all. The upper four were made "active," that is, were accompanied by secretion of saliva. The lowest one was made "inactive," that is, whenever it was stimulated, no food was presented. Suppose, for the sake of example, that stimulation of each of the upper four for thirty seconds is accompanied by the production of 10 drops of saliva, while that of the fifth gives none. Stimulate the inactive spot, and then, thirty seconds later, stimulation of any one of the upper four spots will be found ineffective. At one minute interval, activity begins to return and in order from above down. Thus, the following numbers of drops were obtained, 5, 3, 1, 0. After two minutes, 10, 8, 5, 2. After three to four minutes, 10, 10, 10, 4; and complete return to normal after five to six minutes. Inhibition, therefore, spreads over a wide area of the analyser and disappears from the more distant parts first.

Those conditioned reflexes in which the *time element* enters are also very instructive.. Suppose that the sound of a bell is not at once accompanied by the presentation of food, but only after two minutes' interval; and that this succession is repeated until the conditioned reflex is formed. It is then found that the sound of the bell is not at once followed by secretion, but only after the interval of two minutes has elapsed. Now, during the time between the stimulation and the reflex, it is clear that something must be going on in the centres, but that its manifestation is inhibited. This can be shown by the application of some external indifferent stimulus during the interval, when the saliva immediately appears. It is to be understood that this indifferent agent is not one that produces secretion

of itself and, in fact, if it is presented along with the active stimulus, the effect of the latter is inhibited. It is to be supposed, therefore, that when it causes secretion in the above experiment, it must inhibit the process which was itself inhibiting the appearance of saliva. We have *inhibition of inhibition*, as described above (page 416). A variation of this experiment was made as follows. It was actually done owing to a misunderstanding of instructions. A metronome beat and the giving of food occurred together every ten minutes. Afterwards the signal alone was given. If it occurred at ten minutes' interval, saliva appeared, but not if at shorter intervals.

A conditioned stimulus can be made an inhibitory one. Suppose that a sound and a light are made, each for itself, active, that is, associated with presentation of food, but that, when both occur together, no food is presented, that is, the combination is made inactive. Then one must inhibit the other. Moreover, one may be presented alone and be followed by secretion, and, while the active stimulus is still present, the other, also active by itself alone, may be presented. The secretion stops, because the combination of the two is inactive.

Pavlov (1912, p. 329) states the following rule with regard to the spread of stimuli in the cortex. As they arrive, they spread at first and irradiate, then collect together, fix and concentrate. This law shows itself very clearly in the phenomena of inhibition (1912, p. 330). Suppose that a number of various stimuli have been made signals for activity of the salivary gland. They act also when combined together. But if one of them is made feeble by inhibition, the others are also extinguished, if tested at once. But if the test is not made until several minutes later, it will be found that the activity has returned to all, except to the one previously inhibited, which remains for a much longer time ineffective.

Since, during a conditioned reflex, the whole of the cortex, except the part in action, is inhibited, it follows that, if the stimulus is not followed by presentation of food, so that internal inhibition of this part also takes place, there is a tendency to total inhibition and to sleep.

Sleep itself, indeed, may be associated with food and be excited by a conditioned stimulus. A lullaby may be regarded as the conditioned stimulus to send a child to sleep.

Removal of an area of the cortex damages *permanently* any conditioned reflex in which that area had been concerned. It was found that when a certain large skin area had been made a conditioned stimulus for the feeding reflex, the removal of parts of the frontal lobes abolished the conditioned reflex from a particular, sharply defined area of skin. On stimulation of the ineffective skin area, there is, however, a strong *inhibition* of the effect from an active area. It leads, also, very quickly to drowsiness and sleep. Removal of occipital lobes prevents the obtaining of conditioned reflexes from individual visible objects, although it is still possible from various intensities of illumination. Other cases might be given in which parts of the brain had been removed, resulting in abolition of the power of obtaining certain kinds of conditioned reflexes, but retaining that of others. An animal might thus be spoken of as an idiot, incapable of education, so far as certain systems were concerned, but rational in other systems.

It will be clear, from some incidental facts mentioned, that an opportunity is presented for the investigation of the phenomena of *hypnosis* and of *sleep*. As a working hypothesis, we might suppose that hypnosis is associated with a condition of *active* inhibition, *sleep* as that condition of inactivity of the parts of the brain associated with consciousness which follows on inhibition, if no further excitatory stimuli are supplied. It may be regarded as a zero state, neither excitation nor inhibition; all excitatory stimuli being first removed by inhibition, which itself then also disappears. See also Petrov (1916) and Pavlov and Woskressensky (1916).

If the cerebral cortex is completely removed, no conditioned reflexes can be formed at all. It appears, then, that the cortex is the organ for appropriate adjustment to the varied combinations and changes in the outer world.

As regards the *methods* to be used in these researches, it will be obvious, from what has been said as to the interference of chance phenomena with the establishment of a conditioned reflex, that a properly fitted laboratory, in which all extraneous stimuli are excluded, is a necessity (Pavlov, 1911).

See also the paper by Anrep (1920) on the use of the method for testing auditory sensibility. In this paper the general method is described and various incidental facts, such as "inhibition of inhibition," are referred to.

Pavlov's address given at the Physiological Congress at Groningen (Pavlov, 1913), and his paper (1912) should be read. A research by Orbeli (1909) may be referred to as an example of some of the kinds of work done already in Pavlov's laboratory.

Many of the facts described above were given me by Prof. Babkin and Dr Anrep, who had taken part in numerous experiments.

Conditioned reflexes have been obtained in the snail (Thompson, 1917).

SUMMARY

The contrast between the effects produced by reflex stimulation of nerves and those produced by direct stimulation of efferent nerves is due to the passage through synaptic membranes in the former case.

The increased delay in the case of reflexes is not owing to a need of time to make the synapse conducting, nor for "amœboid" movement of cell processes into contact, but to actual passage through a resistance.

The discharge does not, as a rule, cease when the stimulus ceases, but lasts for a varying time afterwards. This *after-discharge* can be cut short by inhibition, and very sharply.

Repeated subminimal stimuli are capable of evoking a reflex ultimately. In some cases, as that of the scratch reflex, a single induction shock, however strong, will not do so.

Two reflexes making use of the same final common path may reinforce one another. This, together with inhibition, plays an important part in reactions to "constellations" of stimuli.

If the skin area for the scratch reflex is stimulated at two different points with subminimal intensity, *both* stimuli act on the *whole* centre and produce the reflex by "immediate spinal induction."

After a period of inhibition, there is frequently an increased excitability, which is not shown when the stimulus is merely removed for a period equal to that of the inhibition. This *rebound* is not, therefore, due to rest alone.

The synapse between an axis cylinder and the cell body or dendrites of another neurone will only allow impulses to pass in the direction named, and not from the cell body back to the axis cylinder of another neurone. This has been proved experimentally in the case of motor neurones, but there is evidence to show that the synapses of dorsal root fibres (sensory) with cells in the spinal cord is permeable in both directions.

The phenomenon of the refractory period is well marked in reflexes. In the case of the motor neurones used as final common path by the scratch reflex and also by the flexion reflex, the long refractory period must be situated in some neurone on the afferent side, since the latter reflex is not rhythmic.

When the contraction of a group of muscles, necessary for a particular reflex, can be opposed by that of an antagonistic group, it is found that along with contraction of the one group there is relaxation, by inhibition of the centre, of the other group. This is the phenomenon known as *reciprocal innervation*, and was appreciated by Descartes in the case of the eye muscles. The seat of the inhibitory component of the reflex appears to be either at the synapse with the motor neurone of the final common path, or in an intermediate neurone very close to this.

In some cases, where smooth muscle is the effector, there is reciprocal innervation of peripheral origin. Thus, stimulation of the efferent nerve to the muscles causes contraction of one muscle and inhibition of its antagonist. The claw of the crayfish and the dilatation of the pupil may be mentioned.

Under natural conditions, a reflex arc is played upon by various afferent

impulses, some inhibitory, and the discharge depends on the algebraic sum of the whole, as shown in *double reciprocal innervation.*

When the relative intensities of the excitatory and inhibitory stimuli are very nearly balanced, a *rhythmic* discharge results. In the case of the leg, this is alternating on the two sides, so that stepping is produced. Explanations suggested will be found in the text.

The effects of strychnine and of chloroform in converting inhibition into excitation, and vice versa, show themselves in reflexes dealt with by reciprocal innervation. This action is not exercised on the final common path itself, but either at the synapses of different neurones with it, or in some previous synapses, as in fact follows from the seat of the inhibition itself, as stated above. The effect of the conversion of inhibition into excitation by tetanus toxin in willed movements and the suffering it causes is described in the text.

When reflexes use the same final common path for different purposes, they may either reinforce or inhibit each other. Even if they both excite, they may not be able to use the motor neurones at the same time, if they use them in a different way. The *proprio-ceptors* of a muscle are of use in cutting short one reflex and preparing the arc for another.

There are numerous *compound* reflexes, in which the result of one sets another into play.

The intermediate synapses of a reflex arc are comparatively easily *fatigued*, whereas the motor neurones themselves are not so. This fact is of value in preventing the occupation of a particular arc by one reflex for too long a time. Recovery is fairly rapid. A reflex may cease, therefore, either from fatigue or by inhibition, and there are differences between the two cases by which they can be recognised.

Reflexes from *nocuous* stimuli are prepotent. Their receptors are probably free nerve endings. The stimulus required is comparatively large, as is appropriate to the purpose of the reflex.

The mechanism of *autotomy* in the crab is described in the text.

The difference between spinal reflexes and those in which the higher centres, and especially the cerebral cortex, take part is the regularity of the former and the ease with which the latter are modified or abolished by events in other parts of the central nervous system. For this reason, Pavlov calls the former "unconditioned" and the latter "*conditioned*" *reflexes.*

Notwithstanding this fact, Pavlov has devised methods by which conditioned reflexes are amenable to experimental investigation and obtained many valuable results.

The two fundamental mechanisms involved are those of *temporary association* and that of the "*analysers.*"

By the former, a stimulus presented several times in conjunction with an unconditioned stimulus, such as food in the mouth, has ultimately the effect of exciting salivary secretion when presented alone. It has become a conditioned reflex and presents the opportunity for testing the effect of various other stimuli, or events in the external world, on cerebral phenomena.

The "Analysers" are the sense organs, together with their central connections.

The production of a conditioned reflex may be expressed in one way by saying that the reflex arc has taken on a new afferent neurone. But it must be remembered that the connection with this neurone is very easily broken or inhibited, and is modifiable in various ways.

Inhibition is, in fact, the most common fact met with, as in the experiments of Graham Brown and Sherrington on cortical stimulation, as described on page 480. It may even show itself as *internal inhibition*, when the proper consumma-

tion of the conditioned reflex, that is, food, fails to be presented after a number of repetitions of the stimulus for the conditioned reflex.

Other interesting examples of inhibition will be found in the text.

Certain forms of conditioned reflex obtain the mastery over nociceptive reflexes, contrary to the case of spinal reflexes. Thus, a painful stimulus can be made into the sign for a conditioned reflex, and then ceases to be painful.

Removal of portions of the cortex affects permanently the possibility of conditioned reflexes in which these portions take part normally.

The bearing of the facts of inhibition on hypnosis and sleep is pointed out.

LITERATURE

Spinal Reflexes in general.
Sherrington (1906, pp. 1-268).

Irreciprocal Conduction.
Fröhlich (1909, 2).

Reciprocal Innervation.
Sherrington (1909, 2).

Rhythmic Reflexes.
Graham Brown (1912). Forbes (1912, 2). Sherrington (1913, 1).

Conditioned Reflexes.
Pavlov (1910, 1911, 1912, 1913).

CHAPTER XVII

RECEPTOR ORGANS

Organisms of all kinds maintain their existence by adapting themselve in the world of things surrounding them. They depend upon it fo and must avoid, or defend themselves from, other organisms which for food.

As we have seen, nerve fibres themselves can be excited by v of stimuli, chemical, electrical, mechanical, and so on. But it wil that an organism is the better equipped the more delicate its s the actions of the outer world. Nerve fibres themselves, in fact, re powerful stimuli to excite them. Contrast, for example, the force stimulate a delicate organ of touch, such as the human skin, with th to stimulate the trunk of the sciatic nerve of the frog.

Fig. 159. Free nerve endings.

Intra-epithelial nerve terminations in the larynx. Golgi method. On the left, stratified epith right, ciliated columnar epithelium. n, Nerve fibres in the corium.

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NOCUOUS STIMULI

It is natural to suppose that stimuli of an injurious nature woul the first to be responded to in the course of evolution. We notic is reason to believe that free nerve endings are sufficient for the of these stimuli, since it is not desirable that a stimulus, too weak t should evoke a reaction of flight. In fact, we actually find tha endings are present in the skin (Fig. 159).

THE CHEMICAL SENSE

The earliest animals arose in the sea, and would naturally be great variety of chemical substances dissolved therein. Parker (191 the skin of fish is sensitive to acid, alkali, and salts, also to quinine. that it is the hydrogen and hydroxyl ion concentration of the two fi the active properties. It requires a considerably higher concentrati chloride than of hydrochloric acid to stimulate these receptors, alth concentrations used, both would be almost completely dissociat number of chlorine ions would be proportional to the concentratio sodium hydroxide is more powerful than sodium chloride.

We may also take note that the concentration of acid or alkali required, al is very much higher than that to which the differentiated receptors in the hum sensitive. This fact suggests that it is free nerve endings that are stimulat

the irritant action of the substances named. This view is confirmed by the fact that twice molar sugar, which cannot be supposed to be nocuous, has no effect.

The senses of *taste* and *smell* are higher developments of this primitive chemical sense. Delicate special receptor organs have been formed, especially in the land vertebrates. That of smell has, indeed, become a kind of distance receptor, of great importance in many animals, owing to the stimulant substance being conveyed by air or water. It might be thought that the name of smell should be confined to the appreciation of vapours, but it must be remembered that, before acting on receptors, these vapours must enter into solution in the liquid covering the receptors. There is, therefore, no sufficient reason to make a distinction between this sense in the shark and in the dog. It is obvious, however, that transmission through the air by currents is more rapid than through water, and that, for this reason, the growth of the mechanism as a distance receptor, becomes more obvious in land animals. The nature of smell as a distance receptor in fishes is discussed by Parker and Sheldon (1913).

The sense of *taste* is much less delicate than that of smell, and cannot be said to play a great part in the growth of the higher nervous systems. It has scarcely any distance element, even in its most developed form.

It is remarkable that, even in the higher vertebrates, the sensory neurones of smell receptors have retained their primitive condition of cell body in the epithelium itself with nerve processes passing into the central ganglion. But it seems doubtful whether this fact altogether justifies the view suggested by Parker (1912) that the sense of smell represents the ancestral chemical sense. The epithelial cells would very early form chemical products by the action of external chemical agents, products of such a nature as to stimulate the free nerve endings between the epithelial cells and thus form a common basis from which the more delicate mechanisms of smell and taste have been developed. At the same time, we must be careful in limiting these activities to purely chemical ones, since it is difficult to imagine any chemical property common to lead acetate, saccharin and glucose. The acid taste, apparently, is merely a question of hydrogen ion concentration, and it is not difficult to suppose the existence of some peripheral chemical substance very sensitive to this factor.

TOUCH RECEPTORS

In addition to the action of chemical substances in the water surrounding them, primitive organisms are exposed to contact with other objects. Such contacts probably, at first, acted as nocuous stimuli merely, of a nature indistinguishable from one another, but evoking the powerful nociceptive reflexes.

It is curious that, according to Cohnheim (1912, 2, p. 112), the molluscs known as "Heteropods" are insensitive to the presence of food until it comes into contact with them, apparently being unable to appreciate it by chemical sense or by vision, although they possess eyes. After biting an object, however, they appear to recognise by taste whether it is fit for food or not.

From the varied effects produced by substances in contact with the skin, the elaborate system of skin receptors, as we know it in ourselves, has been differentiated. But before we proceed further to the consideration of the higher sense organs, and especially of the distance receptors, a few additional introductory remarks of a general nature are necessary.

THE RECEPTOR MECHANISM IN GENERAL

Organisms, as remarked above, are the better provided for their adaptation to changes in their environment, the greater the number and variety of external phenomena which they are capable of appreciating. To be aware of things happening at a distance gives opportunity for preparation to meet them before they actually arrive. Hence the great advance in animal organisation with the development of organs, such as the eye and the ear, which enable things at great distances to impress themselves.

It is also very necessary that many events occurring in the organism itself should be made known to the nerve centres. We have, as well as extero-ceptors, intero-ceptors, as they are called by Sherrington, and, amongst these, the proprio-ceptors are of great importance. These are the receptors in an active organ which give information to the centres of the state of activity of this organ, and are thus

the means of influencing it reflexly. We shall see the part played by these receptors in discussing "plastic tonus" in the next chapter.

An illustration may assist here. Suppose a general commanding a battle in which several regiments are engaged and extending over a wide area. It is clear that, in order that a particular movement may be effectively ordered, it is necessary for the general to know that the previous order has been carried out. The message may, perhaps, not have reached the body of troops in question. Again, since we judge of the distance between one inaccessible object and another by movement of the eyes, the centres must be made aware that the eye muscles have actually executed the movement intended; knowledge of the sending of an efferent impulse is not sufficient of itself, for the nerve may have been unable to conduct it.

As regards the variety of external forces for which sense organs have been evolved, it is not to be forgotten that there may be forms of energy for which we have no appropriate receptors. Moreover, it is possible that other animals than ourselves may be able to appreciate phenomena of which we are unaware. Quantitative differences of this kind are familiar. We have no appropriate receptors for ultra-violet light. The cat is able to hear notes higher in pitch than man can, and so on.

Further, a form of energy which we know by physical experiments to be one and the same, such as radiation from the sun, is known to us as light or as heat, according to the particular receptors on which it falls, although the only difference is in rate of vibration. More precisely, a particular, somewhat narrow, range of rate of vibration or wave length, appears to the eye as light, longer or shorter wave lengths are unappreciated; but the long waves which have no effect on the retina are able to stimulate certain receptors in the skin and are then called heat.

We see thus, that, in order that a particular form of energy may be able to produce a propagated disturbance in the receptor neurone, what is necessary is that a mechanism of some sort shall be present in which changes shall be produced of sufficient magnitude to excite the nerve fibres, although the incident energy itself may be far too small to do so if it acted directly on the nerve. How small an amount of energy is able to produce such a nerve impulse, if it acts through an appropriate receptor, is shown by the calculation of V. Henri et Larguier des Bancels (1911, p. 856), who found the retina to be sensitive to an amount of light energy as small as 5×10^{-12} ergs. This is about three thousand times as sensitive as the most rapid photographic plate. These considerations naturally lead us to expect that the nature of the receptor organ will vary greatly according to the particular form of energy to be detected; just as we use a galvanometer to detect electrical currents, a thermometer for heat, an actinometer for light, and so on. It is also instructive, in view of the facts to be considered in the next paragraph, to remember that it is possible to detect and measure all forms of energy by converting them into electric currents. Heat, by the use of a thermopile as receptor, sound by a telephone, ordinary kinetic energy by using it to turn a dynamo, chemical energy, as that of the neutralisation of a base by an acid, by the use of the hydrogen electrode, or indirectly by conversion to heat and then by the thermopile, can all be converted into electric currents in a wire, just as in our receptor organs of sense they are converted into nerve impulses. There is, however, a difference to be noted; in the sense organ a small incident energy is caused to set free a larger amount of energy by what we may call a "trigger" action; as if, in our physical instruments, we always made use of what is known as a "relay," such as is done to magnify the energy of the electrical waves received in wireless telegraphy, where a minute amount of energy is caused to complete the circuit of an independent battery, and to set in action a much greater quantity of energy, which can readily be detected.

Muller's Law.—We saw in Chapter XIII. that all evidence points to the fact that there is no difference between forms of nerve disturbances. Impulses may follow one another at different rates, within the limitations of the refractory period, but the individual impulses are in all cases alike, with the single exception of those that follow immediately in the wake of a previous disturbance. Even here, the first disturbance is a normal one and those that follow only differ from it in magnitude. This being so, impulses produced in the optic nerve by light on the retina are identical with those produced by sound in the auditory nerve, by

touch in the skin nerves, and so on. It is obvious that the differences recognised by the organism must be due to "analysers" in the central nervous system. Something has already been said on this point in discussing Pavlov's conditioned reflexes (pages 503-504), and attention may be called to Fig. 158 (page 504). We meet with the phenomenon again in the form of Müller's law of "specific energies," as he called it (1843, p. 1065). The word "energy" is unfortunate here, since it is used in the sense merely of "endowment" or "property" and Müller himself uses it interchangeably with "quality." The law amounts to this, that, *however excited*, each nerve of special sense gives rise to its own peculiar sensation. Müller also (1826, 1840, and 1843) points out that the same external cause may excite a different sensation in different sense organs. "Sensation is not a conduction of a condition of certain external bodies directly to consciousness, but the conduction of a state of a particular nerve, and the excitation of each nerve of special sense has its own peculiar sensation, which cannot be replaced by that of any other nerve."

Sir Chas. Bell (1823, 2, p. 304), in a less clear manner, called attention to the fact that stimulation in any way of a nerve of special sense always gives rise to the sensation which is normally associated with the appropriate stimulation of the receptor organ at the end of the nerve. Fire, he says, gives no sensation of heat when applied to nerves, except to those of the skin or other surface. The retina, pricked by a pin, gives no tactile sensation, but a flash of light. Whatever may be the nature of the stimulus, pressure, vibration, heat, electricity, applied to a nerve, the perception evoked is related to the nature of the end organ, not to that of the stimulus acting on the nerve.

The fact that sensation of light is evoked by section of the optic nerve is often brought as proof of the statement before us, but, since a slight pull on the retina very easily excites the receptors there, it is, by itself, only evidence that the mechanical stimulation of the retina, as well as that by light, can produce the special sensation. The best proof is that afforded by the trunk of the chorda tympani nerve as it passes through the tympanic cavity, as referred to above (page 388). A sensation of taste is produced by either mechanical, chemical, or electrical stimulation at this point.

Why these nerve impulses, alike in themselves, give rise to such widely different sensations when they arrive at their respective cerebral terminations is a matter beyond the scope of physiological analysis. We have to make use of the fact, to a certain extent, in the investigation of the laws of action of the peripheral receptors.

The next point to be noted is that, however these receptors themselves are excited, whether the stimulus is one for which they are specially adapted or not, the sensation evoked is the same. Thus, there are certain spots in the skin which give rise to a sensation of heat, even when excited electrically or mechanically, while there are others which give rise to a sensation of cold when similarly excited. The important point is that specialised receptors are much more sensitive to their appropriate stimulus than to any other. An electrical stimulus strong enough to excite a heat spot excites also a cold spot or a pressure spot, whereas a warm body is able to excite a heat spot when its stimulus is too weak to excite any other kind of receptor. A warm surface thus stimulates only the heat spots, until its temperature is raised sufficiently high to be nocuous and to cause pain. It is possible that the inappropriate stimulus needs to be strong enough to excite the actual nerve fibres themselves, in which case we are clearly dealing with Muller's law itself.

Weber's Law.—As this law is of general application, it may properly be referred to here. It refers to the increase of the sensation, as related to that of the stimulus, and states that, in order to produce a just detectable difference in sensation, an equal fraction of the stimulus must be added to it, whatever its value. Suppose that we could just detect the difference between 10 g. and 11 g., it would be necessary to add 100 g. to a kilogram before the change was noticed. Thus to increase a weak sensation by a given amount requires a less addition to the stimulus than to increase a strong sensation by the same amount. The law is a particular aspect of a law frequently met with in natural phenomena.

It may be put in another form. To excite a series of sensations differing by equal increments, the stimuli must increase in geometric proportion. If

the logarithms of the stimuli are plotted as abscissæ, and the sensations as ordinates, a straight line is produced. Victor Henri et Larguier des Bancels (1912) point out that the law applies to vision in the middle of the range of stimuli only. The curve, drawn as above, with logarithms as abscissæ, is, as a whole, of an S-shape. Similar relations between stimulus and effect apply to the electrical changes in the retina, according to the results of De Haas, and also, as regards the middle region, where the curve is practically a straight line, to the action of ultra-violet light on *Cyclops*, as studied by Mme. Victor Henri et Victor Henri, whose work will be referred to in a future chapter. The conclusion to be drawn is that, in all probability, Weber's law rests on physiological phenomena as its basis.

THE RECEPTORS IN THE SKIN

We pass on to refer, somewhat briefly, to certain facts regarding the different kinds of sense receptors.

Owing to its interest and importance and partly, perhaps, on account of the comparative ease with which it can be investigated up to a certain point, this branch of physiology has produced as much work as any other, probably more. Certain individual sense organs, as the eye and the ear, have large textbooks and memoirs devoted to them alone, or even to particular aspects of them, as, for example, the refractive properties of the dioptric system of the eye. I must be content, therefore, with referring the reader to some of these memoirs for the greater part of the information available (see the list of literature at the end of this chapter).

It is well known that a variety of sensations are obtained by means of the *skin*. Heat and cold have been already referred to; these were shown by Blix (1884) to arise from distinct spots, as also the sense of pressure. Von Frey (1894) showed that there are also distinct pain spots. To the latter investigator we owe most of our knowledge on the question, and his article (1913) may be read with profit. Von Frey's use of delicate hairs, the degree of pressure exerted by each being determined by the weight required to bend it, should be mentioned.

It is impossible to give, at present, an adequate explanation how small differences of heat and cold are magnified sufficiently to excite nerve fibres. Various possibilities might be mentioned, such as a chemical reaction greatly accelerated by heat or some physical mechanism making use of expansion to cause pressure. Pressure stimuli themselves may perhaps be increased by some kind of lever action, as in the case of the long bristles forming the whiskers of the cat, which seem to be sensitive even to air currents, since it is difficult otherwise to suggest an explanation of their guiding power in the dark.

The various modifications of the sense of touch are brought about by combination of movement with contact, by which a series of sensitive points is excited, or the same spot by a series of stimuli occurring at different rates.

In this latter connection, the possibility may be referred to that a nerve fibre may have synapses with two neurones and that the refractory period in one synapse may be longer than in the other. Thus, a slow rate of stimuli may pass both unaltered, while a rapid rate will pass only one unaltered, being reduced to a slow rate in the other one. In this way, there seems to be an indication of the way one nerve fibre might serve to convey impulses giving rise to different sensations. An economy of nerve fibres might possibly be brought about, a point of importance in connection with the very fine gradations in the higher senses, but it is purely hypothetical. There is another point to be remembered here. The roughness of a surface appears to be the same although the finger is moved over it at different rates. This fact indicates that it is not merely the different number of stimuli affecting the nerve ending in a given time that conditions the nature of the sensation, but that this is combined with the sensation of movement given by the muscular sense. So that doubt is cast on any interpretation which assumes that the absolute number of stimuli per second is a controlling factor in different species of sensations.

Protopathic and Epicritic Sensibility.—This is the appropriate place to refer to the experiments of Head, Rivers, and Sherren (1905) on the time of regeneration of various skin sensations. Head caused to be divided the radial nerve (a purely sensory nerve) in his own arm and observations were then made on the returning sensibility. The results led him to regard the sensations derived from the application of stimuli to the hand to be of three different groups: protopathic, epicritic,

and deep. The last group is derived from receptors in the deeper structures and appears to have given rise to some error in previous experiments on the time of regeneration of sensory fibres. The whole of the deep structures in the hand, for example, are not supplied by the same nerve. The *protopathic system* is common to the whole body. It regenerates more rapidly than the epicritic system and is of a more primitive nature. The position of the point stimulated cannot be recognised and a widespread, radiating sensation arises from it. The receptors do not appear to be as sensitive as those of the other types but, when excited, the effect is a powerful one. In the skin, they respond to painful stimuli and to extremes of heat and cold. The receptors of *deep sensibility* answer to pressure and to movement. The fibres from the limbs run in the motor nerves. The system of Pacinian bodies is associated with deep sensibility. The *epicritic system* is a highly differentiated one and regenerates very slowly. It is only found in the skin and endows it with sensibility to delicate touch, with the power of localisation of stimuli, of distinguishing two points and of discriminating fine degrees of heat and cold, together with the other forms of special sensation. It is obviously the system connected with the development of specialised receptors and the intellectual powers resulting from them. Full details will be found in the paper by Head and Rivers (1908). Page May's article (1909) may also be consulted.

SMELL

It is impossible to make a distinction between the differentiated chemical sense of water animals and that of smell in land animals. We are, therefore, justified in regarding the delicate appreciation of the neighbourhood of certain kinds of objects, an endowment which can be shown to be independent of sight and possessed by so many marine animals, as a form of the sense which we know as smell.

The great enemy of the scallop is the starfish, so that, as soon as the near presence of a starfish is recognised by the mollusc, its curious swimming movement is executed by this latter and it escapes beyond reach of its enemy. Although the scallop possesses numerous well-developed eyes around the edge of the mantle, stimulation of these by the shadow of the enemy is not sufficient to excite flight, for the reason which we shall see presently. But W. J. Dakin (see von Uexküll, 1912, p. 329) showed that a small quantity of an extract of starfish, dropped by a pipette in the neighbourhood of a scallop, causes an immediate swimming away. This is a clear instance of the differentiated chemical sense which we may call smell.

A detailed investigation of the sense of smell in higher vertebrates has been made by Zwaardemaker (1902). It may become of a most extraordinary delicacy, thus: $\frac{1}{100}$ mg. of mercaptan in 230 cub. m. of air can be detected, and, of course, only a few cubic centimetres of this air are necessary for the production of the sensation, so that somewhere about 1×10^{-8} mg. is sufficient to excite the receptors for smell.

The paper by Parker (1913, 2) may be consulted with regard to the analogies between taste and smell. The latter is the more sensitive.

PHOTO-RECEPTORS

Radiant energy, from the sun or similar source, may be absorbed by the skin, converted into heat and thus excite heat receptors. But light waves have also a powerful effect in producing many chemical reactions, and these chemical changes may be such as to stimulate special end organs. Further, as we shall see in more detail in Chapter XIX., which may with advantage be read before the present section, any particular chemical reaction is produced only by a certain group of wave lengths, so that the possibility is presented of distinguishing between light of different wave length, that is, a sensibility to colour.

It is probable that appreciation of light and darkness by some photo receptor, sensitive to a photo-chemical change in a substance with which it is in contact, would be the first to appear. By this means the proximity of food or enemy would be recognised, although the aid of some other receptors, probably those of a chemical sense, such as smell, would be required to distinguish between the two.

The sea anemone appears to possess photo-receptors of this simple kind (see von Uexküll, 1909, p. 71), and according to Parker (1903 and 1905) the power is also present in the skin of fresh-water fish, such as the Ammocœte, and in numerous amphibia (see also the monograph by Nagel, 1896). Parker does not think that the elaborate eye of the vertebrate has been formed from this primitive sensibility of the skin to light. The receptor mechanism of the vertebrate eye is, embryologically, an outgrowth from the central nervous system, so that it seems more probable that the photo-receptors concerned may have been formed in the central nervous system itself of a transparent animal.

However this may be, it is obvious that a mere sensibility to light and shade is of comparatively little value, until a mechanism is developed by which images of external objects are formed on a sensitive surface composed of a multitude of elements connected with separate nerve fibres. By this means a picture is, so to speak, conveyed to the brain.

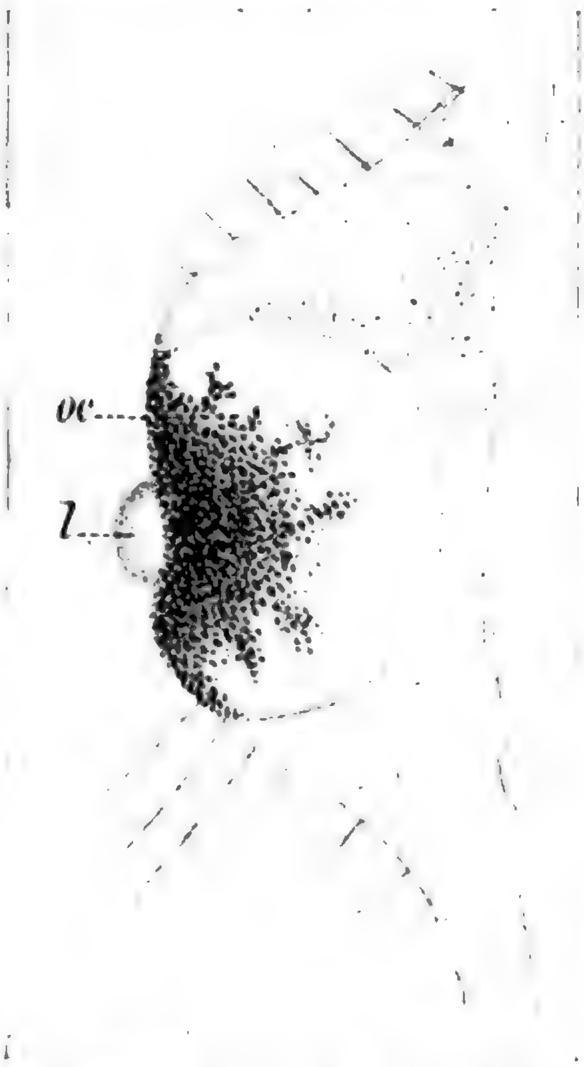


FIG. 160. OCELLUS OF LIZZIA KOELLIKERI.—Seen from the side. After treatment with dilute osmic acid. Obj. F., Oc. 2.
l, Lens. (After Hertwig.)
oc, Receptive mechanism.

A fully developed eye consists, then, of some dioptric mechanism, corresponding to the lens of a photographic camera, together with a layer of a photo-chemically active substance, like the silver bromide of the plate. In the eye, we have also endings of a large number of nerve fibres, attached to complex receptors, which serve to produce nerve impulses from the photo-chemical changes. This part is called a retina. It is also necessary that stray light should be kept out by an arrangement like that of the camera bellows and the dark slide. This is done by pigment cells, which absorb the light. Fig. 160 shows a simple eye. The complex structure of the retina of the vertebrate may be seen in Fig. 161 (from the monograph by Ramón y Cajal, 1894). It is to be remembered that the peripheral receptor mechanism proper consists of the rod and cone, and perhaps of the pigment layer; the other layers, as will be seen from the figure, consist of neurones, interposed between the actual receptor neurones and the nerve centres. The retina thus consists in great part of nerve centres, owing to its mode of development. In the eye of the Cephalopod, which is a highly differentiated one, similar to that of the vertebrate, these intermediate neurones form a distinct ganglionic mass, outside the eye itself. In these organisms, also, the light impinges directly on the receptors, the retina not being inverted, as in the vertebrate, where the incident light passes through the nerve layers before reaching the rods and cones.

Eyes of the simple kind described above (cups of pigment and a lens) exist even in unicellular organisms (Mast, 1916) and in Medusæ (Fig. 160), but it is doubtful whether such simple dioptric mechanisms can do more than serve to concentrate the light on the sensitive cells. In Pecten, we have a number of eyes of an elaborate nature, shown in Fig. 162, from Dakin's monograph (1909). There are here a number of separate nerve fibres, and, in consequence, the possibility of an appreciation of something approaching an image. It is interesting to note that the arrangement noted above in the vertebrate retina, namely, the passage of the light through the nerve layer before reaching the sensitive substance, is also met with in Pecten. The fact suggests that there may be some reason for

the arrangement, in addition to that usually given, namely, the formation of the nerve layer by invagination of the front hemisphere of a spherical outgrowth.

From the work of von Uexküll (1912, p. 329) it appears that, whatever image may be formed on the retina of Pecten, no response is called forth until the object moves. Further, the movement of any object excites the same response, which is a protrusion of the long tentacles, endowed with chemical and tactile sensibility. The object of this response is obviously to obtain further information, and flight results if it turns out that the object is an enemy. Otherwise, flight would be a waste of energy.

When dioptric apparatus is present of sufficient accuracy to form a clear picture on the retina, some mechanism is obviously necessary to adjust the focus for near or distant objects. In land animals, the chief refracting surface is the curved cornea, since the refractive index of the aqueous humour on the inner side of it differs more from that of air than that of the lens does from those of the liquids in which it is immersed.

This can readily be shown by observation on the eye of an albino rabbit. Owing to the absence of pigment, the image of a distant window with cross-bars can easily be seen through the outer, sclerotic, coat of the eye-ball. If a microscope slide be held in such a position as nearly to touch the cornea, and a drop of physiological saline solution be placed between the cornea and the glass, the image disappears, since the refracting surface is now a plane one. On removing the glass, the image reappears.

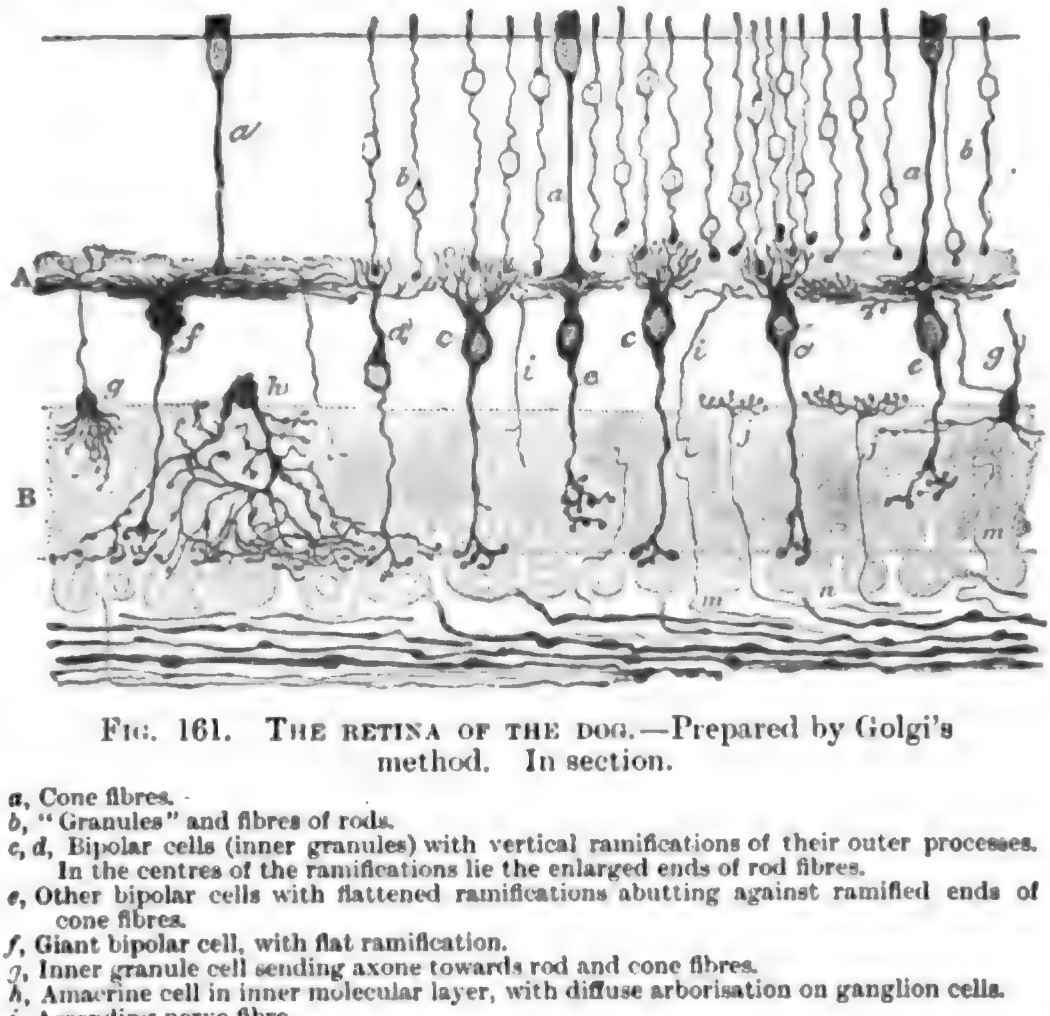


FIG. 161. THE RETINA OF THE DOG.—Prepared by Golgi's method. In section.

a, Cone fibres.
b, "Granules" and fibres of rods.
c, *d*, Bipolar cells (inner granules) with vertical ramifications of their outer processes. In the centres of the ramifications lie the enlarged ends of rod fibres.
e, Other bipolar cells with flattened ramifications abutting against ramified ends of cone fibres.
f, Giant bipolar cell, with flat ramification.
g, Inner granule cell sending axone towards rod and cone fibres.
h, Amacrine cell in inner molecular layer, with diffuse arborisation on ganglion cells.
i, Ascending nerve fibre.
j, Centrifugal fibres.
m, Nerve fibres which become lost in inner plexiform layer.
n, Ganglion cells which form synapses with the end branches of a bipolar cell belonging to a group of rods.

(Ramón y Cajal, 1894, Taf. V., Fig. 2.)

In fish, therefore, the lens has to do the chief work in the formation of an image. Accordingly, we find that its curvature is very much greater than in land animals, the lens being nearly spherical in shape.

In land animals, the chief use of the lens is to adjust the focus of the dioptric system. The curvature of the cornea is not made to change. According to the work of Beer (1898-1901) *accommodation* to near or distant objects is effected in two ways. The first is that present in invertebrates, in vertebrates up to and inclusive of amphibia, together with certain snakes, and consists in the actual change of position of the lens, just as in the photographic camera. The second mechanism is found in a few snakes, in tortoises, lizards, crocodiles, birds and mammals, and consists in a change of the curvature of the lens. In its natural position in the eye, the lens, which is elastic, is focussed for distant objects, owing to the way in which it is pulled upon by the ligaments holding it in place, which cause its curvature to be a flatter one than that assumed when released from tension. But,

by the contraction of a ring of muscle, the ciliary muscle, this tension of the ligaments is released, like that of a stretched cord of india-rubber would be if the attachment of one of its ends were pulled nearer to that of the other end. In consequence of the release of tension, which admits of degrees, the lens assumes

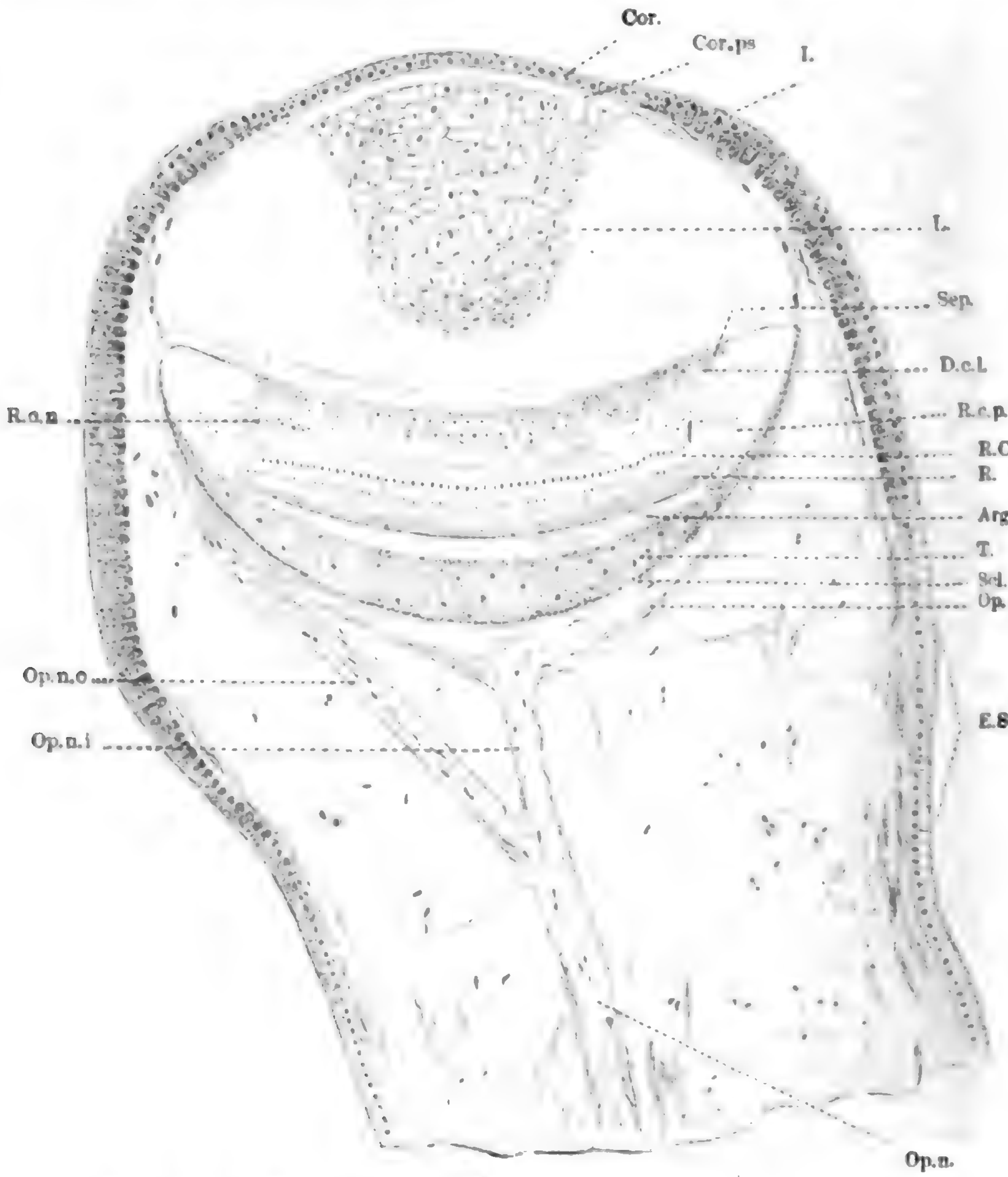


FIG. 162. EYE OF PECTEN MAXIMUS.—Longitudinal section. Magnified 150 times.

Cor., Cornea.
Cor.ps., Pseudo-cornea.
I., Iris.
L., Lens.
Sep., Septum.
D.c.l., Distal cell layer.
R.c p., Pseudo-rod cells.
R.C., Rod cells of retina.
R., Rods of retina.
Arg., Argentea.
T., Tapetum.
Scl., Sclerotic.
Op.n., Optic nerve.
Op.n.i., Inner branch of optic nerve.
Op.n.o., Outer branch of optic nerve.
R.c.n., Nuclei of rod cells.
E.St., Eye stalk.

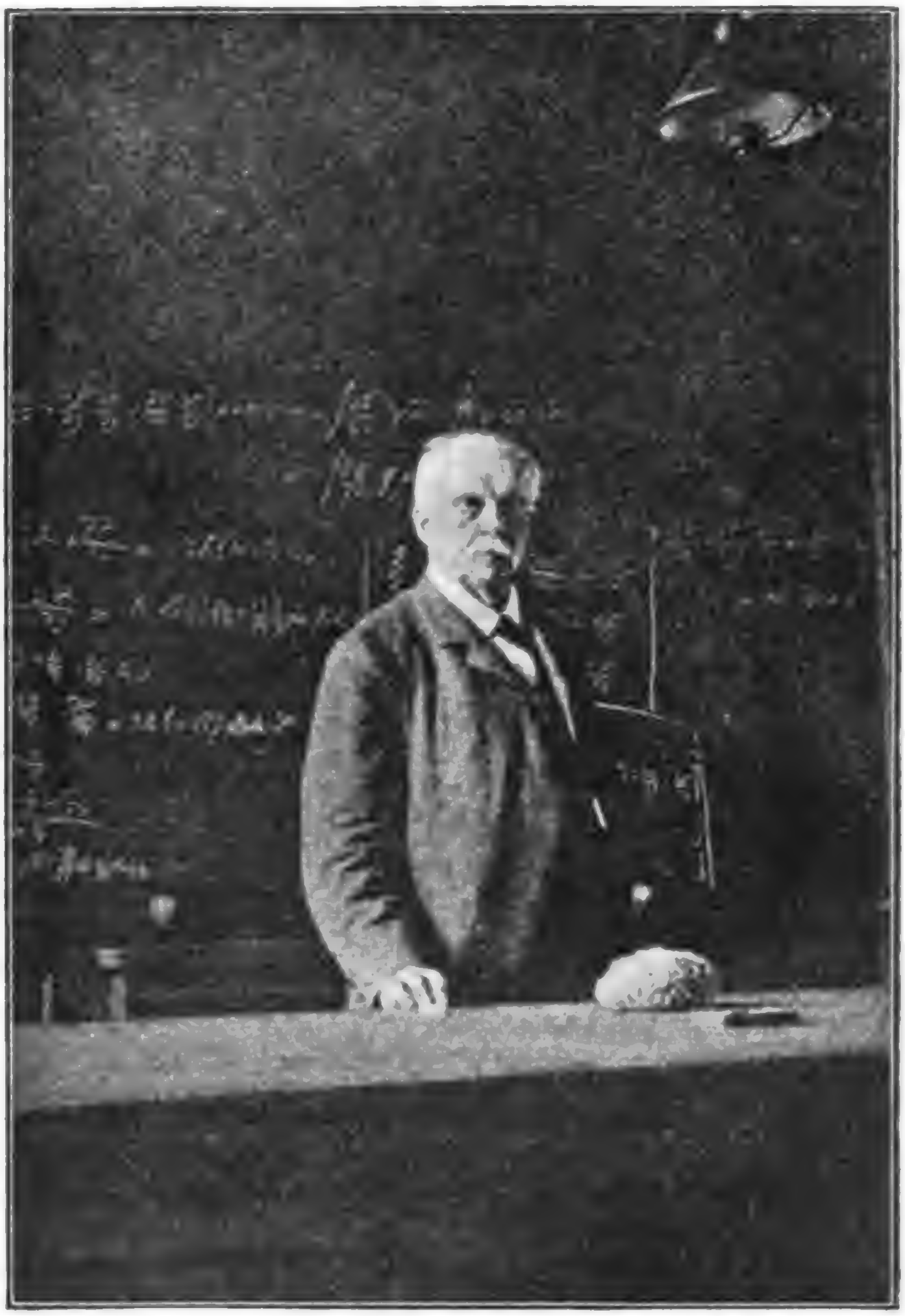
(Reproduced from W. J. Dakin's monograph on "Pecten," by permission of the Liverpool Marine Biology Committee.)

more or less the form it takes when free, that is, a more spherical one; hence it is able to focus near objects on the same plane on which it previously brought distant ones to a focus.

At this point, I may stop for a moment to mention that this mechanism of accommodation was first made clear by Helmholtz, to whom we owe a very large part of our knowledge of the eye as well as of the ear. Although we have already

met with his name in connection with several other fundamental phenomena, such as the doctrine of energy, the electrical double-layer, the rate of the nerve impulse, and so on, this is perhaps the most appropriate place to call attention to his portrait, which will be found in Fig. 163 (given by the kindness of Dr J. T. Bottomley, of Glasgow). His two books on "Physiological Optics" and on "Sensations of Tone" remain the standard works on the subjects with which they deal. A third edition of the former has been brought out (1909-1910) by Gullstrand, von Kries, and Nagel.

The function of the *dioptric system* of the eye is essentially a question of geometrical optics. It can be satisfactorily treated by the Gauss method of reduction to certain refracting surfaces at definite distances apart. Details may be found in the textbooks; that of Parsons (1901) and the article by von Rohr (1909) may be mentioned. We must pass on to the consideration of the phenomena which have been found to occur in the retina in response to stimulation by light.



H. v. Helmholtz.

FIG. 163. PORTRAIT OF HELMHOLTZ.—Taken in his laboratory on 7th July 1894.

(By kind permission of Dr J. T. Bottomley.)

Movements of Cones.—Slow movements of the cones in the frog, brought about by contraction of the long fibres attached to their bases, were described by Van Genderen Stort (1887) (see Fig. 613, p. 522, of Schäfer's "Essentials of Histology"). They appear to result from a reflex, since light entering the other eye causes retraction of the cones in the eye which has not been exposed to light. The effect is also produced by light on the skin, by injection of strychnine and by local electrical stimulation. It is difficult to see what is the function of this movement. It has been suggested that it may be a relic of an ancestral state in which the photo-receptive cells of the epidermis were connected directly to contractile cells, although, if we accept the view of the origin of the eye in nerve centres, there are obvious difficulties in this interpretation.

The *Pigment Cells* of the retina are also excited to movement by the action of light, and here again the use of the phenomenon remains problematical.

The "*Dermatoptic Function*," described by Raphael Dubois (1892), is of interest, as it shows the possibility of light absorbed by pigment acting as stimulus. The siphon of the mollusc, Pholas, is sensitive to light and is retracted when light falls upon it. The response is due to the presence of pigmented cells in the epithelium, prolonged into contractile fibres. According to Dubois, the reception of the light stimulus results in contraction of the fibre, which contraction then, in some way, stimulates nerve fibres going to centres and thus setting off a reflex contraction of the siphon.

Steinach (1892) states that pigmented muscle cells are to be found in the iris and that these cells can be seen, under the microscope, to contract when light falls upon them. The observations were confirmed by Guth (1901).

Changes have been described in the *Ganglion cells* of the retina, but these clearly must be regarded as effects of prolonged stimulation on the cells of nerve centres.

The Visual Purple.—There is every reason to believe that the means by which light stimulates nerve endings is through a photo-chemical reaction. There are an enormous number of chemical reactions which are affected by light, and, of these, one is known in connection with the retina, namely, the changes in the "visual purple." Whether this is the only one we cannot say with certainty, but we shall see presently that its properties are in extraordinary coincidence with certain aspects of vision. As will be shown in Chapter XIX., if a substance is sensitive to rays of a particular wave length, such as would be necessary to account for colour vision, it must absorb these rays. Since they must be in the region of the visible spectrum, the substance must have an absorption band in the region referred to, and, therefore, be itself possessed of colour. Although visual purple is the only such substance detected as yet in the retina, with the exception of certain coloured globules, which are not sensitive to light, described by Kühne (1878), in the cones of birds, it is conceivable that others may be present in the small amount required, and even in the requisite number, to account for the number of colours to which the eye is sensitive. It is, moreover, not impossible that visual purple, as obtained, may be a mixture of a number of different substances, each with an absorption for a particular group of wave lengths and giving rise to its own particular photo-chemical product, to which a definite receptor only is sensitive. More probably, in the formation of the particular photo-chemical product, molecular vibrations of a certain rate are excited, possibly by resonance, with the excitation of receptors by the energy set free.

Although it had been known for some time that the retina of a frog, removed in the dark, appeared to be of a red or purple colour, when observed in the light, and that the colour disappeared more or less rapidly, the definite association of the pigment in question with visual sensation was not made until Boll's work (1876), followed by the more extensive and detailed work of Kühne and his fellow-workers (1878).

The colour of the pigment is not exactly what most people would call purple, it contains much more red. But, having a trace of violet in it, it is best described as a deep pink or rose colour.

It is bleached by light, but, in the retina, the colours return in the dark. Whether there is new pigment formed or whether the products of the action of light return to their original state in the dark, a very common phenomenon in photo-chemical reactions, is not altogether certain. It appears, however, that under some conditions, solutions of the pigment recover their colour when allowed to stand in the dark after being bleached by light. It has no isolated absorption band in the spectrum, but absorbs light almost equally in all parts, leaving a little red and violet, hence its colour. It is to be expected, then, that it would be responsive to light of all wave lengths, except the extreme red and violet. As indicated above, a series of substances with absorption bands along the course of the spectrum, when mixed together, might give a similar continuous absorption.

Visual purple is found in the rods only of the mammalian eye, in the so-called

cones of birds, and in the corresponding structures of the retina of the frog, fish, and cephalopod. Since it is not present in the cones of the human eye, it is absent from the region of sharpest vision, the *fovea centralis*, a fact which has led some observers to doubt whether it has any real importance. Edridge-Green, however, has brought forward evidence to show that it diffuses into the fovea from the surrounding rods. The rods, themselves, he regards as being concerned only with the formation of the pigment and not receptor organs for light. As regards this last point, it appears that the sensibility of the various zones of the retina in the recognition of form is directly proportional to the number of cones per unit area which they contain. Put in another way, the images of two points are recognised as distinct according to whether they fall on two cones or not, so that they must be further apart to be recognised as two in the peripheral parts of the retina, where the cones are further apart. But the microscopical appearance of the cell connections of the rods is very similar to that of the cones and does not suggest that of secretory cells only.

Kühne showed that the pigment is sensitive to light while in the eye, and that photographs of objects can be made on the retina owing to this fact. These are called *optograms* (1878, p. 225).

The difficulty of obtaining information as to the *chemical nature* of visual purple, as it has become the custom to call it, is obvious, on account of the very small quantity to be obtained. Its solubilities are peculiar; according to Kühne, it is only dissolved by bile salts with readiness. This fact suggests a colloidal suspension; the lowering of surface tension produced so powerfully by these substances would facilitate a great dispersion of the particles. It does not, in fact, diffuse through parchment paper, so that the bile salts can be removed by dialysis. In addition to dispersion of the pigment, the bile salts appear to disintegrate the rods. The pigment is not attacked by trypsin, so that it is not of protein nature. The method used by Kühne to obtain his purest preparations will be found on p. 454 of his paper with Ewald (1878), and on p. 266 of his article in Hermann's "Handbuch" (1879).

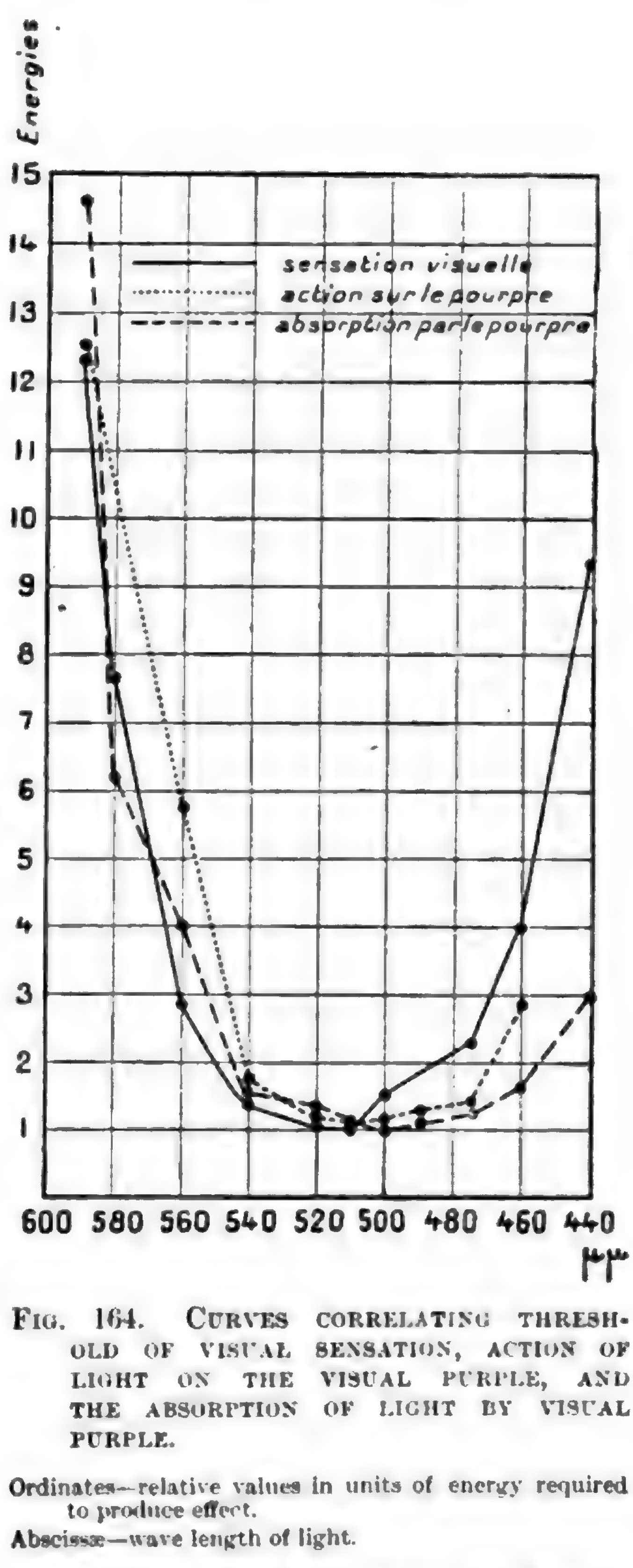


FIG. 164. CURVES CORRELATING THRESHOLD OF VISUAL SENSATION, ACTION OF LIGHT ON THE VISUAL PURPLE, AND THE ABSORPTION OF LIGHT BY VISUAL PURPLE.

Ordinates—relative values in units of energy required to produce effect.
Abscissæ—wave length of light.

(Victor Henri et Larguier des Bancels, 1911, 1, Fig. 4.)

When light falls on the peripheral parts of the retina in man, it is found that, when diminished so as to be just visible, it is only that part of the spectrum between wave lengths 600 and 440 $\mu\mu$ (orange to blue) that is visible at all, and the sensation is one of light only, without colour, whatever the wave length

used. Now Victor Henri et Larguier des Bancels (1911, 1) have determined the amount of energy just sufficient to excite (threshold energy), the bleaching effect on the pigment and the amount of light absorbed by it, all at various wave lengths between the values named. When put into curves, these three factors are found to follow the same course (see Fig. 164). This means that to produce the same sensation, by different wave lengths, requires such an amount of radiant energy that the amount absorbed by the visual purple is the same in all cases. This is a powerful argument in favour of the participation of the pigment in vision, at all events in that particular form of the sensation investigated. The same investigators find that the absolute quantity of energy required varies with the duration of action according to a complex law, which seems to result from a combination of that of excitation of nerve with that of a photo-chemical reaction. If the energy quantum be worked out by the formula in Nernst (1913, 255, etc.), it is 2×10^{-12} erg for the D line, practically the same as the limit of the sensibility of the retina (page 512 above). This sensibility is then the maximum possible.

Electrical Changes.—Holmgren (1880) noticed that the incidence of light is accompanied by an electrical change in the retina, and further work was done by Dewar and M'Kendrick (1876), Kühne and Steiner (1880), Waller (1900), Gotch (1903 and 1904), Einthoven and Jolly (1908), Piper (1905, 1910, and 1911), Fröhlich (1913), and others. Although, when the interpretation of these results is better understood, they will undoubtedly help in the explanation of retinal processes, it must be confessed that, at present, they do not throw much light on the question. The main fact is that, in the uninjured eye of the vertebrate, the incidence of light causes an electrical change in such a direction that the nervous layer of the retina becomes electrically positive to the rod and cone layer. This state, with some subsidiary waves, lasts during the illumination, and disappears again, at a certain rate, when the light is removed. But, immediately after removal of the illumination, and before the effect produced by it has disappeared, a puzzling further change, in the *same* direction as that produced by the incidence of light, makes its appearance for a short time. Apparently darkness causes a temporary effect of the same sign as light does. When, however, the curve is carefully analysed, as can be done with that obtained by the capillary electrometer or the string galvanometer, it is found to have a complex form, which Einthoven and Jolly and Piper have resolved into a compound of three different curves of different time course, and it is important to note that, by proper construction of each of these components, it is possible to obtain a curve like the original, including the "dark" effect, from curves which have an *opposite* direction in light and in darkness. This fact disposes, indeed, of some of the difficulty attending the dark effect, but seems somewhat artificial, since no explanation can yet be given of the meaning of the three hypothetical processes. It is true that the Young-Helmholtz theory supposes that there are three fundamental components in visual sensations, red, green, and violet. When in certain relative proportion, the sensation of white light results and that of various colours according to the relative proportion of the three components. But Gotch's experiments (1904) showed that the electrical response to red, green, or violet light was of the same form in each case, although differing in latent period and magnitude, while each showed a similar rise when the stimulus was removed. The three hypothetical components of the curve, assumed by Einthoven and Jolly and by Piper have, as it seems, nothing to do with the three fundamental sensations of the Young-Helmholtz theory. We shall see other reasons later why this theory cannot be regarded as a satisfactory one. Still less do the facts of the electrical change support theories such as that of Hering, where colours which are complementary to one another, that is, which make white on mixing, are supposed to cause opposite changes in the "visual substances," the one anabolic, the other catabolic. If this were so, red and violet should give electrical changes of opposite sign.

As examples of actual experiments, Fig. 165 (according to Piper, 1910) may be of interest. The electrical change in the mammal is of a simpler nature than that in the bird. Piper also shows (1911, Figs. 38 and 39) that the response is a simple one in the retina of the Cephalopod, a fact which indicates that a part

of the complexity of the vertebrate effect may be due to the nervous elements of the retina.

If we regard it as probable that the electrical response is connected with a photo-chemical reaction, we may consider for a moment such a reaction as that of the decomposition of silver chloride by light in the simplest conditions, so that the products are not removed from the sphere of action by other reactions. In the dark, we know that silver and chlorine reunite to form the chloride again, and at a rate controlled, in the main, by mass action. As soon as light begins to act on the chloride, a portion is decomposed and the products begin to recombine again owing to their own affinity and independently of the action of light. It will be clear, therefore, that, according to the intensity of the illumination, an equilibrium will be established at such a position that the rate of recombination is equal to that of decomposition. As soon as illumination ceases, recombination begins at its own proper rate, since the opposing reaction no longer exists. Further particulars of such photo-chemical changes will be found in Chapter XIX., and we shall see that there are other reactions which would illustrate the point more accurately, since the decomposition of silver salts by light is not quite so simple as assumed above. It serves, however, to illustrate the nature of the apparent equilibrium

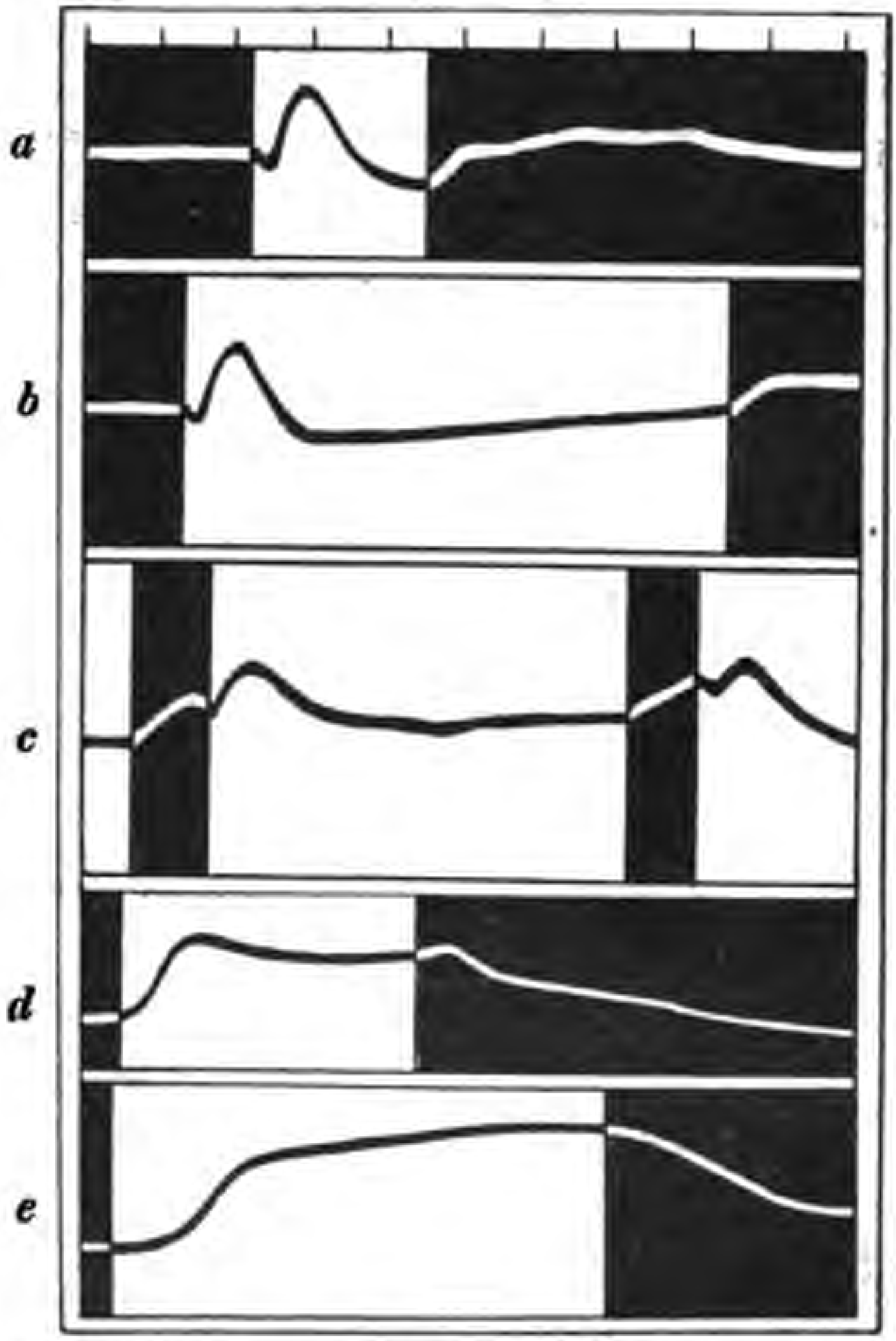


FIG. 165. ELECTRICAL CHANGES PRODUCED BY LIGHT IN THE RETINA.—String galvanometer. The white areas are periods of illumination, the black areas are periods of darkness.

Time in 0·1 second at the top.

a, Eye of pigeon. Brief illumination (0·23 second). Negative and positive variations with light, positive with subsequent darkness.
b, Eye of pigeon. Longer period of action of light (0·72 second).
c, Effect of brief periods of darkness (0·1 second each) on the pigeon's eye previously exposed to continuous illumination.
d, Eye of rabbit. Illumination for 0·5 second. Positive variation with light and secondary slow rise. Small, slow negative variation, after short latent period, with subsequent darkness.
e, Eye of Cephalopod (Eledone). Simple positive variation with light, after latent period of 0·023 second; remaining practically constant during illumination. Return to original value with darkness, also after a latent period.

(After Piper.)

attained under the action of light, which is only kept up by the continuous supply of light energy. (See Bauer, 1911, on the regeneration of visual purple, which continues during the action of light.)

It may be mentioned that Brossa and Kohlrausch (1913-1914) have found that the form of the electrical response varies with the wave length. The work of Fröhlich (1913), also, on the eye of the *Cephalopod* requires consideration. This eye presents certain advantages as regards the question before us. As already pointed out, the nervous elements are situated in a ganglion outside the eye itself. Although the retina is very highly developed, the visual elements are of one kind only, similar to the rods of the vertebrate retina. It has visual purple and the receptors are exposed directly to the light rays. Fröhlich confirms the result of Piper that the electrical response is less complex than that of the vertebrate. But the chief interest of his work consists in the demonstration of the fact that the retinal electrical response is not a steady one, but consists in a series of rhythmic waves, from 20 to 100 per second, being more rapid the more intense the illumination. These waves are also to be seen on the return of the curve after cessation of the stimulus, but of a lower rate. There is no indication of a "dark" response in the same direction as that on illumination. The effects of red and of blue light were also compared with that of white and the interesting fact found that red light required to be increased enormously more than white or blue to give the same increase in electrical effect, as shown in the table:—

Electromotive Force in Millivolts.	Relative Intensity of Light.		
	White.	Blue.	Red.
Minimal	...	1	20
0·2	...	5	1,020
0·4	1·25	11·2	12,500
0·6	5	80	...
0·8	80	12,500	...

With the same intensity, the rate of the rhythmical changes is less with red than with blue. Whatever may be the significance of the vibratory nature of the electrical change, it is clear that it does not represent the actual rate of vibration of the light itself, but it does not appear to me that the author's conclusion that the red end of the spectrum is exciting, the blue end inhibitory, on account of the rapid rate of the waves produced, is a necessary one. The apparent inhibition produced by rapid rates of stimulation of weak intensity has been discussed previously in reference to Wedensky's phenomenon (pp. 429 and 426).

Colour Vision.—This important question cannot be adequately treated here and the reader should refer to the various textbooks. There are, however, some facts, chiefly brought out by the work of Edridge-Green (1909 and 1911), to which brief reference must be made, because they are only just beginning to receive the attention they deserve.

The Young-Helmholtz theory assumes that there are only three primary colour sensations, red, green, and violet. Now, while it is true that any colour may be formed by mixtures of these in appropriate proportions, it is also true that more than three primary sensations would also serve the same purpose, three is, in fact, the minimum. And it is a matter of universal experience that blue and yellow have just as much right to be considered primary as the other three. In fact, Newton's division of the spectrum into red, orange, yellow, green, blue, indigo, and violet is much nearer the truth. Indigo, however, is rarely seen as a distinct colour. Edridge-Green divides people into classes according to the number of distinct colours they distinguish and shows that there are various degrees of colour blindness according to the number of colours seen in the spectrum. From the point of view of evolution of the colour sense, he points out that it is practically certain that the distinction between different wave lengths, that is, the recognition of a difference between colours, would first show itself at the extremes of the region which is appreciated as light, the region between the

wave lengths 770 and 396 $\mu\mu$ about. Red and violet would be distinguished first, next green between them would be added, finally yellow and blue. Correspondingly, a common form of colour blindness is the tri-chromatic, where red, green, and violet are the only colours perceived. Yellow is called red-green, and blue, green-violet.

A further important point established by this investigator is that, contrary to what a casual examination of the spectrum might lead one to suppose, there is not an infinite series of gradations of colour along the spectrum, but that it can be divided up into a number of patches, each of these patches being of a uniform colour. Thus the eye is not capable of appreciating an indefinite number of spectral colours. The fact can be shown by the use of a spectrometer with adjustable shutters in the ocular. When any part of the spectrum is thus isolated, it is found that a certain breadth can be found which appears to be all of the same colour. Thus the whole spectrum is divided up, by normal sighted people, into some sixteen to twenty monochromatic areas. Houston (1916) treats the theory mathematically.

Edridge-Green has brought out methods of testing colour vision on the basis of the above facts, together with other considerations. These methods are now being accepted as the only reliable ones.

The existence of colour vision in the animals lower than man is obviously a difficult matter to decide. Orbeli (1909), in his work on conditioned reflexes, found the dog unable to form such reflexes to colour alone, merely to differences of luminosity. Later observers found that, with great difficulty, colour can be used in this way. The colour sense must be very rudimentary in the dog. Fröhlich (1913) thinks that the difference between the electrical changes to red and to blue in the Cephalopod indicates colour vision, but since differences in intensity of white are also associated with differences of rate of rhythm, the only evidence is the quantitative one of the rapid diminution in comparative effect as the intensity of the stimulus is increased. The apparent fondness of certain birds for brilliant colours, and, in fact, the general evolution of colour in flowers and butterflies and so on, suggests some sort of colour sense in these animals. According to Polimanti (1915), however, silkworms are colour-blind. Frisch (1914) finds a sense of colour in fishes.

The numerous phenomena connected with positive and negative after-images are beyond the scope of this book. One fact should, however, be noticed, namely, that certain combinations of spectral colours give what appear to the eye to be colours as pure as the spectral colours themselves, but of a different wave length from those of which they are composed. For instance, red and green give a yellow, which is indistinguishable from spectral yellow. This fact is not easy to explain. Hartridge (1912) gives reasons for holding that the effect may be merely physical, so that the yellow-perceiving mechanism may really be excited by the mixture of red and green. (See also Edridge-Green, 1915.)

Mention should also be made of the new apparatus of A. W. Porter, which is the most perfect yet devised for the investigation of colour mixing, after-images and other colour phenomena. This instrument has been shown at the Physiological Society's Meeting, but the description has not yet been published.

Mosaic Vision.—The compound eye of the insect and crustacean is a highly developed organ and is usually considered to act as a series of tubes, with opaque walls, by which that ray only which is a continuation of the axis of the tube arrives at the receptor mechanism. In this way an image is formed. The explanation of the elaborate structures present, some of which appear to be refractile, is uncertain. The monograph by Exner (1891) may be consulted.

RECEPTORS FOR SOUND

In order that the rhythmic vibrations of bodies, which are the material basis of what we ourselves call sound, may excite the ends of nerve fibres, it would seem that the most natural way would be to make use of the principle of *resonance*, which has been described on page 88 above.

If we have a series of strings in regular order as regards their period of vibration, sound waves of a particular period will affect one of these strings only

and set it in vibration. A structure of this nature exists in the cochlea of the internal ear of higher vertebrates and is known as the "basilar membrane." It is true that it does not consist of separate strings, but as it only possesses tension in a transverse direction and not longitudinally, it is only capable of periodic vibration in the one direction. Its transverse measurement is of a regularly increasing magnitude from the base to the apex of the cochlea and the whole organ is coiled into a spiral. The nerve fibres, by means of a complex structure, the organ of Corti (see Fig. 166), are stimulated when that particular element of the basilar membrane which resonates to a given note is set into vibration by it. This is, in brief, the theory proposed by Helmholtz and further details may be found in his book "Tonempfindungen" (1863). In the sixth edition, the description of the theory will be found on p. 232.

Other theories have been suggested, such as the "sound pattern" theory, in which the basilar membrane is supposed to vibrate as a whole, but with "nodes," or lines of rest, in different places according to the pitch of the note. None, however, seems to agree with the general facts of the structure of the organ of Corti as

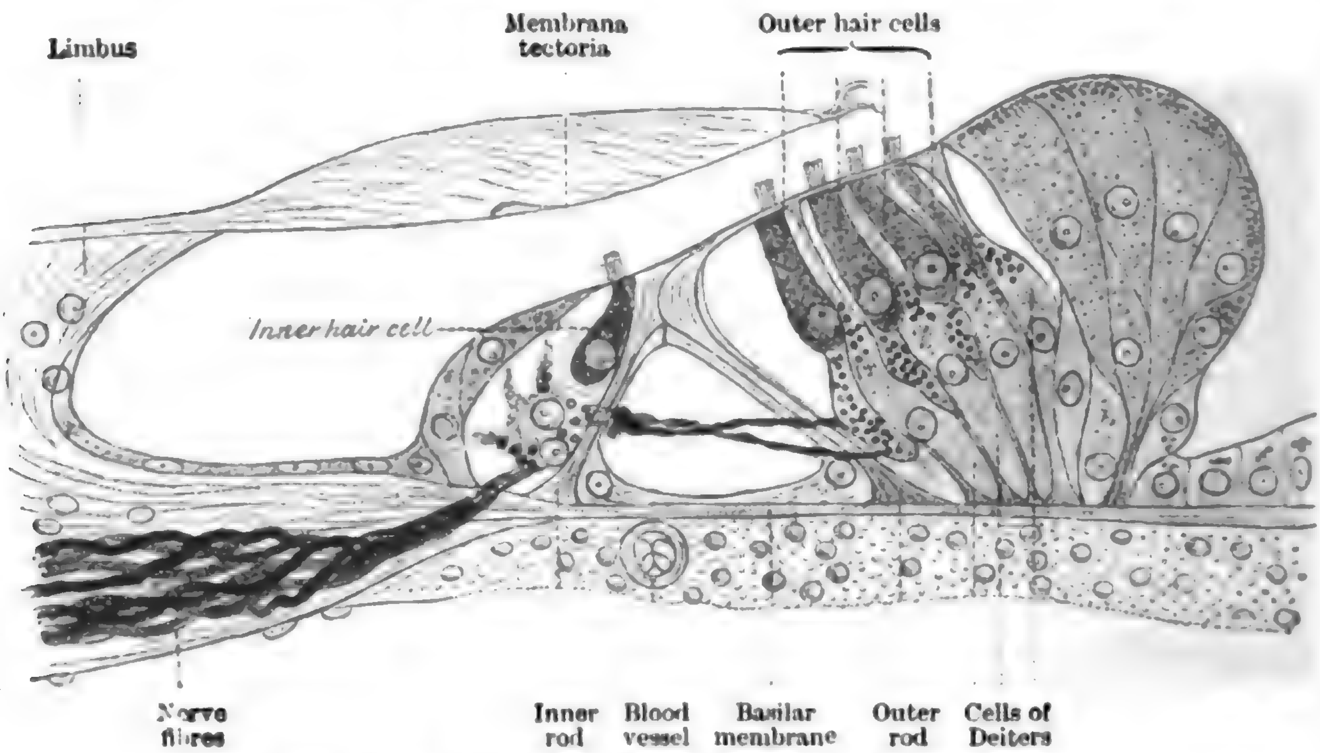


FIG. 166. ORGAN OF CORTI OF MAN.—Magnified.

(Retzius. Schäfer's "Essentials of Histology," Fig. 635.)

well as that of Helmholtz does. This theory has, moreover, recently received a striking confirmation in the experiments of Yoshii (1909). Guinea pigs were exposed to the sound of a particular note on an organ pipe or siren for thirty to forty days in succession. Local degenerations were then found to have been produced in the organ of Corti. These degenerations were in different places according to the note made use of, and were *transverse*, not longitudinal. It is true that the degeneration extended somewhat on both sides of the actual region corresponding to the tone itself, but Helmholtz's theory of resonance would not exclude the possibility of neighbouring portions of the membrane being, to some extent, also set in vibration and it is clear that the nature of the experiments of Yoshii could scarcely afford evidence as to the minimal intensity of sound necessary to cause resonance of a very limited element of the membrane. Secondary changes were also found, in the experiments quoted, in the nerve fibres and ganglion cells belonging to the particular region of the organ of Corti affected by the sound; none in the tympanic membrane nor in the transmitting structures of the middle ear.

The hair cells act as transmitters of the vibrations of the membrane, after these vibrations have been intensified by the structures forming the pillars of the arch of Corti, which rest on the membrane (see Bayliss, 1919, 3, diagram on p. 109).

The *tympanic membrane* is interesting physically. It is so formed, by its shape,

tension, and attachments, as to be "aperiodic"; that is, it has no definite period of vibration of its own, so that it can transmit any rate of vibration indifferently.

The perception of sound seems to have arisen somewhat late in the course of evolution. There is no satisfactory evidence that invertebrates possess it. Of course, the periodic vibrations of a sounding body, if sufficiently strong, can affect the touch receptors of the skin, but, as we know from our own experience, the periodic impulses in the nerves from these organs do not give rise to the sensation of sound; the cerebral "analysers" necessary for the purpose are not brought into play. This fact serves to confirm the view of the indifference of the actual nerve impulses themselves. As to hearing in fish, see Du Bois-Reymond (1917), Parker (1918).

In birds and mammals the auditory organs, as valuable distance receptors, are highly developed, as we saw in discussing the conditioned reflexes of the dog. Their importance when speech, even in its most rudimentary forms, makes its appearance will be sufficiently obvious. In fact, the more or less musical notes made by certain insects, such as the cricket, by the aid of special apparatus, seems to imply the presence of an auditory organ of some kind.

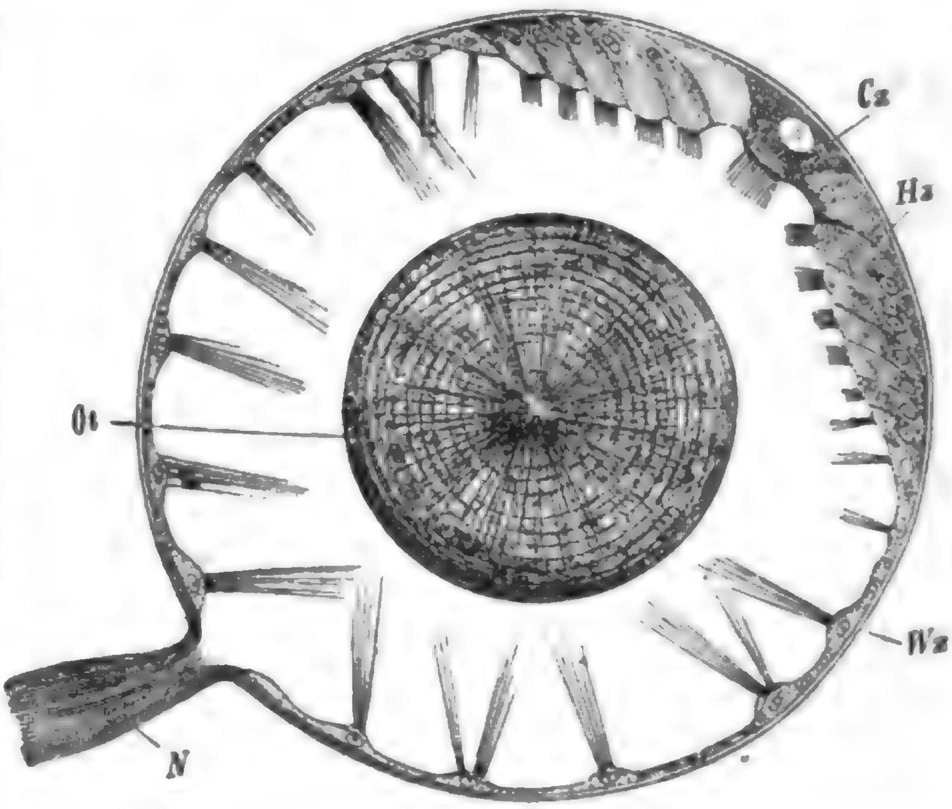


FIG. 167. STATOCYST OF PTEROTRACHEA (A FREE SWIMMING MOLLUSC).

N, Nerve.
Ot, Statolith in the interior of the sac, which is filled with liquid.
Wz, Hair cells on the inner surface of the wall.
Hz and *Cz*, Cells with short bristles, supposed to be the sensitive cells.

(From Claus's "Elementary Text-Book of Zoology." Translated by Adam Sedgwick. London: Swan Sonnenschein, 1884, p. 86.)

POSITION RECEPTORS

Certain organs, present in most animals, even in the Medusæ, were supposed at one time to be connected with the sense of hearing and were called "*otocysts*." These organs consist essentially of sacs, lined with cells, and containing a liquid in which a loose "otolith," or several of them, is freely movable. Nerve fibres terminate in the cells of the sac, and the "otoliths" may be sand particles or any similar substance, insoluble in the liquid.

Although Farre (1843) showed that these organs in the Crustacea act as "delicate antennæ" and have no auditory functions, it was not until comparatively recently that it has been generally recognised that their function is to serve as receptors for the sense of position with regard to the direction of gravity. Verworn proposed that they should be called "*statocysts*" and the solid bodies within them, "statoliths." Beer (1898) showed definitely that Crustacea have no receptors for sound as such.

Fig. 167 shows the structure of a typical "statocyst" from Pterotrachea, and it is plain that the weight of the statolith will rest on different receptor cells according to the position of the animal and thus afford information of its position with regard to the vertical.

An ingenious experiment of Kreidl (1893) neatly demonstrated the fact in Crustacea. As is well known, these organisms periodically shed their outer

chitinous covering as they grow too large for it. Along with it, in some species, the inner lining of the statocysts comes away, naturally taking the statoliths also. These latter must therefore be replaced by new grains of sand or similar bodies. Kreidl placed the animals under such circumstances that the only grains available were iron filings, which were duly taken into the statocysts. On bringing a magnet into various positions with relation to the animals, the iron filings were attracted and pressed against various points of the lining of the statocyst, and the animals showed by their movements that the effect on them was the same as if, under normal conditions, they had been turned into such a position that the weight of the grains would have excited the cells in question.

A detailed account of the properties of the statocysts of Pecten will be found in an article by von Buddenbrock (1911).

Fig. 168. Semicircular canals of man.—Photograph of an enlarged model made by Tramond, Paris. The three arcs are seen to lie in the three dimensions of space. The membranous canals are shown inside the bony canals, which are partly cut away. The cochlea is at the bottom.

In addition to organs of this kind, the vertebrate possesses a remarkable organ, the *labyrinth* or semicircular canals. The statocysts are known in the vertebrate as utricle and saccule. There are three semicircular canals on each side, and from Fig. 168 it will be seen that these loops are arranged in the three dimensions of space. This fact, in itself, suggests that they are concerned with the sense of position, but Crum Brown (1874) and Cyon (1873) were the first to draw attention to the relation. It is, however, to the experiments of Mach (1875) and of Breuer (1891) that we owe the clear presentment of their mode of action. Flourens (1828) had obtained, on section of the semicircular canals, peculiar movements of the head, differing according to the canal injured. It appears that their structure is such as to enable rapid changes of position of the head in space to be appreciated and their particular direction to be known. The receptor cells lining a part of the tube of which each of these loops is composed, and especially those of the dilated ampulla at the end of each, have long delicate hairs projecting into the liquid filling the tube. Since there is free circulation all round the circle, any movement of the head of the animal, to which the base of the hair cell is attached, will move these cells through the liquid, owing to the inertia of the latter not allowing it to share the movement at once. The result of this relative movement is to drag the sensitive hairs through the more or less stationary liquid, bending them and thus exciting the nerve endings at their attached ends.

The investigations of Ewald (1892), chiefly on the semicircular canals of the pigeon, will be found of much interest. They show how the sense of the position of the head in space is disturbed by injury or stimulation of various component parts of the labyrinth mechanism. The recent work of Wilson and Pike (1912) is a valuable contribution to the knowledge of the effects of stimulation and of extirpation of the labyrinth in various mammals.

Rossi (1915) investigated the relative movements of endolymph and labyrinth-walls in Mustelus by making the former visible by particles of lamp-black.

It is not to be supposed that the only information obtained as to the position of the head in space is derived from the labyrinth. The eyes, as well as the proprio-ceptors of muscles, play a large part in the process, these various receptor organs mutually correcting each other. The reader will probably have noticed how the eye is liable to be deceived by passive movements of the body, when these are unnoticed. A railway train rounding a curve deviates from the vertical owing to the "banking" of the track and, if the line is well laid, it is hard for a passenger to convince himself that the buildings which he sees through the windows are not leaning, although he may know that it is the train itself which is out of the perpendicular. The reader should, perhaps, be reminded that this observation is not possible on such lines as those where it is the custom of the guard to inform passengers in the restaurant car when the train is coming to a curve, so that there may be no "slip between the cup and the lip."

The astonishing *sense of direction* possessed by some animals, such as the carrier pigeon, is difficult to explain except as a wonderful memory for labyrinthine sensations received as it is taken from place to place in a basket.

It appears that *gradual* change of position is appreciated rather by the statocyst organs and does not readily excite the receptors of the semicircular canals, which respond to rapid changes to which the relatively inert particles of the former organs would not react sufficiently quickly.

COMBINATIONS OF SENSATIONS

It will have been noticed how sensations from different kinds of receptors are combined together to give more accurate and detailed information of external objects. This is particularly the case with the proprio-ceptors of those muscles which move receptor organs in a definite way, such as those of the eye and the hand, when combined with the sensation derived from these sense organs themselves. In this way the notions of space and so on are formed. But here we pass over to the province of psychology, and it is difficult to avoid the use of such words as "sensation," which imply consciousness, in the description of receptors. The reader must understand that nothing further is to be assumed here than the existence of certain nerve impulses passing to particular regions of the brain becoming connected up with other neurones, according to states present in other parts of the nervous system, and finally giving rise to the activation of some effector.

The discussion of binocular vision and similar aspects of the photo-receptor mechanism is beyond the space permissible here.

RECEPTORS IN PLANTS

Plants, like animals, are in relation with changes in their environment, and have also developed means of intensifying and determining the direction of the action of external forces.

The former mechanism is especially well marked in the so-called "excitable organs," in the narrow sense, where rapid movement exists. The bristles of the leaf of Dionæa are quite entitled to be called receptors; they make the leaf very sensitive to the contact of insects. A similar phenomenon is to be seen in the leaf stalk of *Mimosa pudica*, also in the stamens of various species of Centaurea (the blue corn-flower) and in other situations.

In their sensibility to *gravity*, whose direction they are able to appreciate plants have an actual separation in space of the receptor and effector as there is in animals. It is the point of the growing rootlet that is sensitive, while the response occurs in a region at some distance from this. It may be mentioned here that the proof that the roots of plants are sensitive to gravity was first afforded by Knight (1806), who used centrifugal force to replace gravity and thus obtained a more powerful stimulus. In such cases, it would be quite justifiable to speak of a "reflex action."

As to the mechanism of the gravity receptors, a similar view was arrived at

independently by Haberlandt (1900) and by Nemec (1900). It assumes that each cell of the sensitive tissue corresponds to a statocyst of the animal. In the plant cell, the statoliths are usually starch grains, which fall and form a little heap on the lowest part of the cell. The precise part of the cell thus affected depends on the position of the root or stem as regards the vertical line. In plants in which the statoliths consist of starch, exposure to cold causes them to be used up and the reaction to gravity is abolished until more are formed in warmth and light.

The direction of *light* is appreciated by the leaves of plants, as shown by their setting themselves at right angles to it. Haberlandt (1909, p. 557) points out that, in many cases, the outer ends of the epidermis cells are of a vaulted shape, so that parallel rays of light would be brought to a focus somewhere near the inner ends of the cells. If the axis of the cell is directed towards the light, the middle of the base of the cell is most brightly illuminated, and it is to be presumed that when the brightest part moves to one side or the other a reaction takes place in the stem, the result of which is to bring the bright spot to the centre again. In other cases the cuticle is formed into a lenticular shape. Haberlandt has shown photographically that the light is actually brought to a focus on the inner ends of the cells by these arrangements.

The articles on sense organs in plants by Haberlandt (1904 and 1909) will be found of interest.

SUMMARY

The finer the differences between external forces which an organism is able to appreciate, the better equipped is it to make use of or to defend itself against these forces.

Nerve fibres themselves are not sufficiently easily stimulated by these forces, except in cases where the latter are actually injurious and damage the structures of the organism. There are, in fact, free nerve endings in the skin for the appreciation of such nocuous stimuli.

A mechanism of some sort is therefore necessary to magnify the various minute forces acting on the organism, so as to produce a force of sufficient magnitude to set up a propagated disturbance in nerve fibres. Such mechanisms may be of different kinds, since nerve fibres are excitable by electrical, mechanical, chemical, and other stimuli. These mechanisms are the "receptors."

A primitive kind of chemical sense, allied to taste and smell, seems to be one of the first developed. Touch receptors, to appreciate delicate contact, would also be of early formation.

Events occurring in the organism itself, as well as those of the external world, require to make their existence known to the nerve centres. Hence we have intero- and extero-ceptors. Amongst the former are the proprio-ceptors, by which an organ under the influence of excitation from the centres gives information of its state of activity to the centres themselves.

The distance receptors, such as the eye, ear, and, to a certain extent, those for smell, are the most important in the development of the highest intellectual qualities.

Since nerve disturbances are all of identical nature, whatever be the kind of external energy which acts on the receptor organ, it is clear that the difference between sensations derived say from the eye and the ear, must be due to the arrangements of the nerve centres, the "analysers." This is Müller's "law of specific sense-energies." A nerve fibre of special sense, however excited, always gives rise to the same sensation.

A receptor organ differentiated for a particular kind of stimulus, differs from other receptors, in that it is sensitive to very small stimuli of the appropriate kind, which would be far below the limit of appreciation by a receptor adjusted for another kind of stimulus. The amount of light energy required to excite the

retinal receptors is very small indeed, compared to that of the same form of energy required to excite the heat receptors of the skin, for example.

In the skin, there are receptors for heat, cold, touch, and pain. These are again grouped by the first relay of central analysers into the two groups of protopathic and epicritic sensibility. These two groups also apply to other regions of the body, some regions, however, being possessed of receptors for the protopathic group only. Their more precise definition will be found in the text.

Receptors for light are, in all probability, arranged to make use of a photo-chemically sensitive substance. The products of this reaction, or possibly the changes of energy involved in the course of the reaction, are such as to excite the nerve terminations. Thus we may have a primitive sensibility to light situated in the skin generally.

But, to be of value as a distance receptor, an organ for light stimuli requires to be able to form images of external objects. So that we find a dioptric mechanism present to produce an image on a sensitive surface composed of a number of elements each connected with a separate nerve fibre.

The different methods of focussing this dioptric mechanism, accommodation for near or distant objects, are given in the text.

The only known photo-chemical substance present in the retina is the visual purple. It is sensitive to nearly the whole of the visible spectrum, but whether it consists of one substance only, or of several, or whether other photo-chemical substances are present is not yet known. In order to account for colour vision, the photo-chemical changes produced by light of one wave length must differ from those produced by another wave length, so that different receptors may be stimulated.

That visual purple is, at least, one of the photo-chemically active substances of the retina, is shown by the fact that the light absorbed by it in different parts of the spectrum, the threshold stimulus necessary to produce sensation in the peripheral parts of the retina, and the bleaching effect of the light on the pigment, all follow the same curve. Other properties of the visual purple are described in the text.

There are certain characteristic electrical changes produced by light acting on the retina. The actual curve obtained experimentally is more or less complex, but can be analysed into a compound of three or more simple curves, each of which has an opposite direction at incidence and at disappearance of light. But these components have no connection with the three hypothetical sensations of the Young-Helmholtz theory. Since the electrical change in the Cephalopod is less complex in nature, and the nerve elements in this case are separated from the eye itself, it appears likely that some of the complexity of the electrical change in the vertebrate eye is due to these nerve elements.

There is reason to believe that there are either six or seven primary colour sensations in man, as described by Newton. Further, the whole spectrum does not consist of an indefinite number of gradations of visible tint, but of a series of patches, each of which, when isolated, appears to be of a uniform colour (monochromatic). The number of these areas is about sixteen to twenty in normal sighted people, but the precise number differs according to circumstances.

The mechanism of the receptors for sound consists of a membrane, stretched so as to be in a state of tension transversely only. Its transverse measurement increases regularly from one end to the other, so that it is capable of resonance to different rates of sound vibrations at different regions. The vibrations of the different regions are intensified by the structures known as Corti's organ, in order to be able to excite the nerve fibres of each region. Degenerations can be produced in localised areas by exposure to a particular note for a long time.

The receptors for sound appear to have arisen somewhat late in the course of

evolution. Periodic impulses, if intense, may affect touch receptors without causing a sensation of sound, which requires an appropriate cerebral analyser.

There are certain organs, "statocysts," present in practically all animals from the jelly-fish upwards, and indeed there are similar structures in the higher plants, whose function it is to enable their possessors to appreciate their position with respect to the direction of gravity. This is done by the presence of a loose particle or particles in a sac, which press upon different receptor endings according to the position of the sac. By introduction of iron filings, an animal can be made sensitive to the direction of magnetic force.

In addition to statocyst organs, the vertebrate possesses a system of three canals, the semicircular canals, or labyrinth, on each side. These are arranged to correspond with the three dimensions of space and are capable of detecting rapid movements and appreciating their direction. This is done by the aid of the inertia and internal friction of the liquid filling them. Sensitive hairs, attached to receptor cells on the walls, are drawn through the liquid and bent, owing to the fact that the liquid does not immediately follow the movement of the walls of the tube containing it.

Sensations derived from the labyrinth play a large part in the maintenance of the tonic contraction of the muscles necessary for posture.

Attention is called to the manner in which combinations of sensations from different receptors are used for the purpose of forming complex notions, such as those of space and time, etc.

Plants, also, have developed means of intensifying and determining the direction of external forces, especially those of gravity and of light. The region of a growing root, for example, sensitive to gravity is not identical with that in which the response takes place. The mechanism appears to be the same as that of the animal statocysts, grains of starch being generally the movable particles.

The direction of light is appreciated by the leaves of plants owing to refraction by the outer ends of the epidermic cells, these ends being shaped as lenses. By this means the spot most brightly illuminated on the base of the cell differs in position according to the direction from which light rays enter the epidermis.

Certain plants possess structures very sensitive to touch, and in many cases a rapid movement of an organ results from a slight stimulus.

LITERATURE

Primitive Receptors.
Parker (1903, 1905, and 1912).

Taste.
Nagel (1905).

Smell.
Nagel (1905). Zwaardemaker (1902).

Skin Receptors.
Von Frey (1913). Head and Rivers (1908).

Photo receptors.
Helmholtz (1909-1910). Edridge-Green (1909, 1911, 1919).
Von Rohr (1909) *re* Dioptrics of the Eye.

Auditory Organs.
Helmholtz (1863). Yoshii (1909).

Statocysts.
Von Buddenbroek (1911). Mangold (1912).

Labyrinth.
Ewald (1892). Wilson and Pike (1912).

Plant Receptors.
Haberlandt (1904, 1909).

CHAPTER XVIII

TONUS

This word really implies a state of persistent excitation and is appropriate enough as applied to the condition of a nerve centre when sending out a constant stream of impulses which maintain some effector organ in a state of activity. As we shall see presently, however, it does not so well apply to the case of smooth muscle, which may, as it seems, remain in a shortened state without necessarily being in a state of excitation. Since it is to this case that the name has most commonly been applied, and the last word has not yet been said as to the nature of the process, we may retain the name for both kinds of phenomena. With regard to the history of the name and idea, see Sherrington (1919).

As indicated, there are three sets of phenomena to be taken account of, although perhaps only for convenience of description. (i.) The prolonged state of contraction of smooth muscle, which is automatic, or independent of the receipt of excitatory impulses from nerve centres. (ii.) That shown under certain conditions by the cross-striated, skeletal muscle, of which mention has already been made as "decerebrate rigidity" (page 417), and is dependent on stimuli from the centres, disappearing when these are cut off. (iii.) The state of some nerve centres themselves, in which they appear to give out constantly nerve impulses apart from the receipt of messages from receptor organs. The discharges of such centres are frequently rhythmic, as in the case of the respiratory centre.

We will take first the case of smooth muscle, with its natural "tonus."

TONUS OF SMOOTH MUSCLE OF VARIOUS KINDS

It is a general property of this kind of tissue, wherever met with, to maintain itself in a certain degree of shortening apart from impulses from nerve centres. It is also, almost invariably, provided with two kinds of nerves—a set which increase the tone, excitatory, and a set which diminish it, inhibitory.

This peripheral tonus may also show itself as rhythmic changes, as in the case of the heart muscle. As has been remarked above, this structure behaves as smooth muscle.

In the first place, what is the evidence of a natural, inherent tonus in smooth muscle, omitting the heart for the present?

The Blood Vessels.—Goltz was the first to point out that the dilatation of the blood vessels, which results from section of their constrictor nerves, on account of the cutting off of continuous impulses from the vaso-constrictor centre, is not so great as that produced by stimulation of dilator nerves (Goltz, Freusberg, and Gergens, 1875, p. 62). Thus, after section of the vaso-constrictors, a state of moderate contraction still remains, which can be further reduced by stimulation of dilator nerves. This moderate tonus, left by section of constrictors, increases in a few days, and becomes nearly equal to the original one in some weeks, although the nerves may not have regenerated (Goltz and Freusberg, 1874, p. 175).

In the frog, after pithing, rhythmic contraction of arterioles is present, while the vessels can still be dilated by the action of carbon dioxide (Bayliss, 1901, 1), showing that they were previously in a state of contraction.

In the mammal, after destruction of the spinal cord, although all nervous influence is thereby cut off, the arterial pressure remains at 30 to 50 mm. of mercury.

M'William (1902) found that excised mammalian arteries pass readily into a state of contraction, which seems to depend upon a supply of oxygen. They can be relaxed by carbon dioxide. Similar observations on the effect of oxygen and carbon dioxide were made by Severini (1878, p. 93, and 1881) on the mesenteric vessels of the frog.

Reaction to Stretching.—The denervated smooth muscle of the earthworm was shown by Straub (1900) to respond to stretching by a contraction (see Fig. 132, page 436). The stomach of the frog behaves similarly (Winkler, 1898). The question of the behaviour of the arterioles will be discussed in Chapter XXIII.

The Muscles of the Chromatophores of the Cephalopod.—Hofmann (1907, 3) showed that there are no peripheral ganglia in this case, but that the tonus returns after section of the nerves. He is inclined to attribute it to an effect of carbon dioxide in moderate concentration.

The Adductor Muscle of Anodonta.—Pavlov (1885, pp. 21, 22) showed that the tonus of this muscle is not due to nervous impulses from ganglion cells, since it does not disappear when the visceral ganglion is removed, and there are no ganglion cells in the muscle itself. But stimulation of the nerves from the visceral ganglion to the muscle, after the ganglion itself has been cut out, causes inhibition.

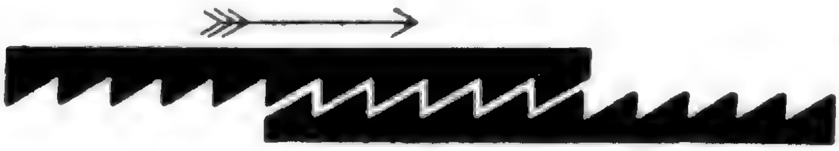


Fig. 169. Diagram to illustrate a catch or ratchet mechanism.—The upper piece can be pushed in the direction of the arrow and the total length of the model shortened in this way. But the upper piece cannot be moved back again, unless the two pieces are intentionally separated from one another by the depth of a tooth.

This reference to the adductor muscle of the Mollusc leads us to consider some noteworthy peculiarities, which are most easily investigated in these organisms, although they seem to be more or less present in all smooth muscle, and perhaps even in skeletal muscle, as we shall see later.

THE "CATCH" MECHANISM IN SMOOTH MUSCLE

I use the word "catch" as a translation of von Uexküll's name "Sperrung," but it is a matter of difficulty to find one which suggests the complete meaning of the German word. Before trying to explain the idea, we will examine a few experimental facts.

The strength with which a bivalve mollusc holds its shells together is known to every one who has tried to open an oyster by merely pulling the shells apart. On the face of it, there is nothing to suggest that this fact may not be due to the reflex contraction of a powerful muscle. It is found, however, that weights may be arranged to pull continuously, and yet the shells remain firmly closed against a considerable force for many days. To take an example, it requires a tension to be exerted by each square centimetre of the adductor of *Dioxinia exoleta* equivalent to the weight of 2,400 g. in order to close the shells against the elastic cushion which forces them open. Yet the animal can do this for twenty to thirty days continuously without evidence of fatigue (Parnas, 1910). Consideration of such facts led Grützner (1904) to suggest that the muscle fibres cannot be exerting tensile stress by a continuous excitatory process, but that the fibres must be "hooked up" in some way, by a kind of arrangement similar to a ratchet, and kept in the position to which the shortening process brought them. If we raise a weight to a certain height and hold it suspended, we have seen that considerable work has to be done all the time and that fatigue soon results. But if a bolt is shot out under the weight, so as to support it, it remains in the raised position without any further expenditure of energy on our part.

The next experiment is one on *Pecten* which I will give in the words of von Uexküll (1912, p. 311). "If one takes a normal Pecten out of the water, it gives two or three flaps with its shells before permanently closing them. While it is open, a piece of wood is pushed between the shells, which then close and hit upon

the wood with so powerful a crash that their edges are splintered. The wood is then held as in a vice. One can, however, pull it out by twisting it about backwards and forwards, and then one is surprised to see that the shells remain motionless, just as would the jaws of a vice if an object clamped between them had been forced out. The shell movement shows not the least degree of elasticity. The muscular fibres seem to have been suddenly frozen solid." If one next tries to *open* the shell, no effect can be produced, but even the pressure of a finger is sufficient to press them *nearer* together, and in this position they remain fixed again, so that they cannot be brought back. The nearest mechanical illustration that can be given is that of two racks with saw teeth, as in Fig. 169; these will glide over one another if pulled in the direction of the arrow, but resist any pull in the opposite direction. The fact that the animal itself can allow the shells to open, shows that the "catch" can be removed by some means. This, as we shall see presently, is done by "inhibition" from the central nervous system. In the model, it might be supposed to be effected by separation of the two racks to the extent of the depth of a tooth. The device of Fig. 170 may perhaps assist in understanding the process. If a flat piece of soft iron be arranged so as to be able to move around an axis at one end, and an electro-magnet fixed at a short distance above it, on sending a current through the coils of the magnet the weight of the piece of iron is raised; but, in order to hold it up, energy must be continually supplied to the magnet, and this energy is dissipated as heat. This part of the process corresponds to the behaviour of the sartorius muscle of the frog in A. V. Hill's experiments (page 450). Suppose, however, that a thin strip of steel spring, with a little projection on it, is arranged at right angles vertically at the free end of the piece of iron, and in such a position that the projection together with the spring can be pushed back by the iron weight as it rises. When the weight has passed over the projecting bit, the spring flies back underneath the weight, and the latter will remain suspended when the current is switched off the magnet. In order to allow it to fall again, a second electro-magnet is fixed at the back of the spring and, when this magnet is actuated, the support is drawn back with the spring and the weight falls. This last mechanism corresponds to the impulses from the central nervous system, which release the adductor muscle of the mollusc.

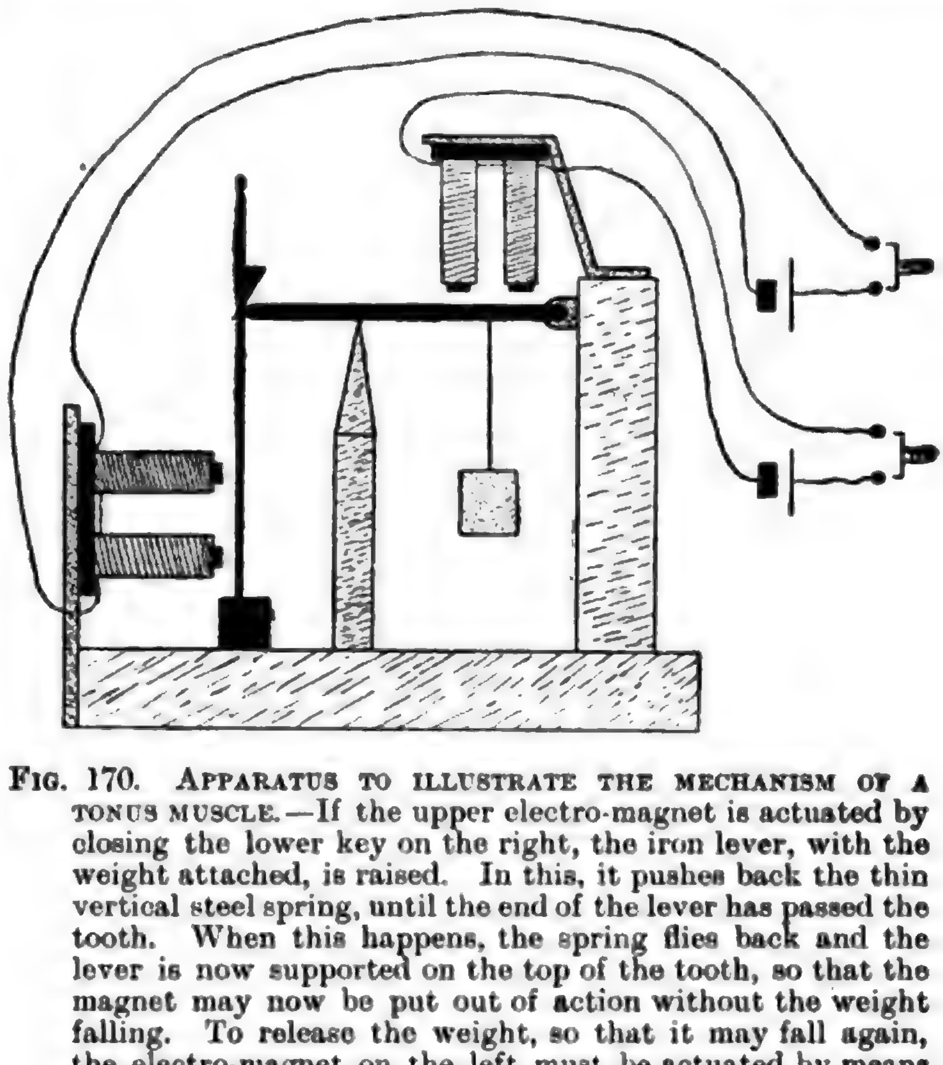


FIG. 170. APPARATUS TO ILLUSTRATE THE MECHANISM OF A TONUS MUSCLE.—If the upper electro-magnet is actuated by closing the lower key on the right, the iron lever, with the weight attached, is raised. In this, it pushes back the thin vertical steel spring, until the end of the lever has passed the tooth. When this happens, the spring flies back and the lever is now supported on the top of the tooth, so that the magnet may now be put out of action without the weight falling. To release the weight, so that it may fall again, the electro-magnet on the left must be actuated by means of the upper key. This attracts the spring and draws away the support from the lever, which then falls until it reaches the pointed support. Finally, the circuit of the second electro-magnet is broken, the spring flies back and the apparatus is in its original state. The two keys may be supposed to be nerve centres.

Now the muscle which has this remarkable property must be able also to

shorten actively and, indeed, if tested by pressing a hard object upon it, it is found to be quite soft when relaxed and as hard as cartilage when in the contracted state.

If Fig. 171 be examined, it will be seen that the adductor muscle consists of two distinct parts, a larger round part, which has a clear appearance and consists of cross-striated fibres, and a smaller more opaque part, of smooth fibres. In order to distinguish them, we may call, with von Uexküll, the former the "motor" muscle and the latter the "catch" muscle. The reason is that, if the "catch" muscle be removed, the motor muscle can be excited to contraction and keeps the shells closed as long as the stimulation lasts; but, as soon as this ceases, the elastic hinge causes them to open again. If the "catch" muscle be cut through while the shell is closed, the other muscle is unable to keep it closed; whereas, if the motor muscle be divided, the shell remains closed. Thus the large motor muscle seems to bring the shells together quickly and for a moment; the smaller catch muscle then holds them fast in the position to which they have been brought by the

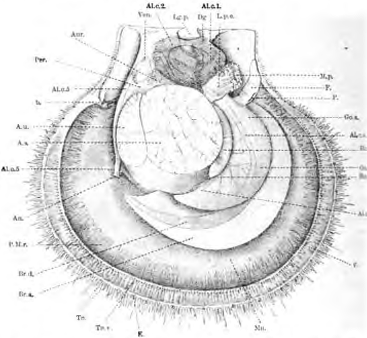


FIG. 171. ANATOMY OF PECTEN.—RIGHT VALVE AND RIGHT LOBE OF MANTLE REMOVED.

Aur., Auricle.
Ven., Ventricle.
Per., Pericardium.
A.u., Non-striated part of adductor muscle.
A.s., Striated part of adductor muscle.
Br.a. and ***d.***, Lamellæ of gills.
Tn., Tentacles.
Tn.v., Velar tentacles.
E., Eye.
Mn., Mantle.
V., Velum.
F., Foot.
Lg.p., Ligament pit.
Al.c. 1 *to* 5, Parts of alimentary canal.

(Reproduced by permission of the Liverpool Marine Biology Committee. For the meaning of the remaining letters, see the original monograph by W. J. Dakin, 1909, pl. 2, Fig. 1.)

contraction of the other. In its contraction it follows up, as it were, that of the other muscle and fixes the whole system at the point where it stops.

Another aspect of the phenomenon is pointed out by Parnas (1910). If we try to pull apart the shells of Pecten by means of weights, we find that, as already mentioned, a very large weight is required to do so. If, however, we hang a considerably less weight on the shells when open, they are unable to close against it. Thus the muscle is able to hold up a weight which it cannot raise.

If the nerves from the visceral ganglia to the muscles are cut through while the shell is open, stimulation of the muscle ends of the nerves will produce contraction but no maintenance beyond the duration of the stimulation. On the other hand, a remarkable fact shows itself if these nerves be cut while the catch mechanism is at work, the shells being closed; there is no relaxation, neither can stimulation of the nerves remove the catch. The catch muscle remains permanently at the length it had at the moment when the nerves were cut.

But there are other nerve cords, one on each side, which connect the visceral ganglia with the cerebral mass, and electrical stimulation of these nerves is able to control the catch muscle in both directions. That of the right side causes its inhibition, so that the shell opens. That of the left side causes shortening and catch action, so that the shell is permanently closed. These various observations are due to von Uexküll, who regards them as a confirmation of his view that "excitation" is not a wave of change passing along a nerve, but something which flows hither and thither like a fluid. He speaks of the tonic state in which the catch muscle remains, when its nerves are cut while in that contracted condition, as the "tonus- or excitation-trap." This view has been criticised on a previous page (page 424).

A corresponding phenomenon is described by Veress (1908, pp. 195-196) in the caterpillar of Cossus, after it has spun its cocoon. If extracted from the cocoon and pinned out, it exhibits rhythmical contraction of the muscles of the body wall. These can be inhibited by touching the cuticle. But the interesting point is that at whatever stage of contraction the muscle may be in at the time, it is fixed at that stage for a certain period. The "inhibition" affects only the rhythmical movements; it does not cause relaxation of the muscle, but merely fixation at that degree of contraction which existed at the moment of the stimulus.

Brief reference may be made to some other cases in which similar phenomena are to be seen. The *spines of the sea urchin* are surrounded at their bases by a circle of about thirty double muscles around each spine. Each of these muscles consists of an inner cord, white and opaque, and an outer one, clear. If we apply a momentary stimulus to the integument near one of the spines, the muscles on that side contract, but only for a moment, and the spine afterwards returns to its original position. If the stimulation be repeated several times, the spine is pulled over and remains so for a considerable time after the stimulation ceases. In this state it opposes much resistance to being displaced. The single stimulus excites only the outer motor muscles; the repeated stimulation excites the inner catch muscles in addition (von Uexküll, 1909, p. 91).

A mechanism similar to that of Pecten has been described by Jordan (1913) in the case of *Holothuria*.

The phenomena shown by *Sipunculus* are very instructive. When the body wall of this tubular animal contracts, it forces out the proboscis, exerting a pressure of about six centimetres of mercury. The proboscis is then cut off, the central nervous system removed, and the remains of the body tube tied on to the end of a glass tube. Sea water is then poured into the tube until its level is about half way up the tube. Then the preparation is immersed in sea water. It is found that to whatever depth the body tube is immersed, the meniscus always remains at the same place; in other words, the capacity of the sac remains constant, although the actual internal pressure must be considerably greater when it is in air than when the weight of the water inside is counterbalanced by that outside. That is, *the muscle fibres can maintain the same length in equilibrium with different pressures.*

This phenomenon is also to be met with in the *urinary bladder* of the higher

vertebrates, according to the researches of Mosso and Pellacani (1882). It was found that the internal pressure which this organ can withstand without further distension is independent of the actual distension existing at a given time. That is, the length of the muscle fibres of its wall may be very different and yet have the same tension; or rather, as we should say, in view of the behaviour of the invertebrate muscle, the fibres may be "hooked up" by a catch mechanism at various degrees of shortening. It will be noted that in this case, as in some others to be mentioned later, the motor and catch mechanisms must be, as far as we know, in the same muscle fibre.

The next question with which we are faced in the consideration of this prolonged "tonic" contraction of smooth muscle is whether the state is associated with any increase of *metabolism* beyond the normal one. If the muscle is held in the shortened position by a catch or ratchet mechanism, it would appear that increased metabolism is not to be expected, or of a much less degree than in tetanic contraction. Parnas (1910) has, in fact, made experiments which show that, in the bivalve mollusc, none is to be detected. He loaded mussels (Anodonta), whose adductor muscles had an area in section of 0·3 sq. cm., with a weight of 3,000 g. for three hours and found no increase in the respiratory exchange, either during or after the loading. Indeed, if one compares the entire respiratory metabolism of these animals, under the conditions stated, with the increase in that of a skeletal muscle of the mammal, also holding a weight of 3,000 g. per 0·3 sq. cm., it only amounts to about 0·00003 of the latter, calculated from results on the entire metabolism in man. The anodon muscle uses 0·008 mg. of oxygen per hour, as compared with some 2·8 mg. for the gastrocnemius of the cat (Verzár, 1912, 1, p. 248). Take another experiment by Parnas on three specimens of *Venus*, which consumed 3·222 mg. of oxygen in four hours, or 0·805 mg. per hour. Loaded with 1,000 g. each for three hours, the consumption was 0·786 mg. per hour, and, subsequently, without load for three hours, 0·811 mg. per hour. A Pecten consumed, at rest, 0·672 mg. of oxygen per hour; under a load of 500 g., 0·679 mg. per hour.

Bethe (1911) investigated the question in another way and confirmed the view of Parnas. He found that no evidence was to be obtained of fatigue nor of loss of weight in fasting molluscs holding up a weight for a considerable time. If the consumption of carbohydrate had been comparable with that of cross-striated, skeletal, vertebrate muscle, an amount greater than the weight of the entire animal must have been burnt up.

An interesting calculation is made by Bethe on the tonus of the arterioles in a mammal. If the mechanism were like that of the skeletal muscle, one-sixth to one-quarter of the whole resting metabolism of the animal would be in the arterioles.

The *hardness* of a muscle is proposed by Noyons and von Uexküll (1911), as a test of the tonic activity of the catch mechanism. In the leech, the same length of animal may in one case be under a tension of 10 g., in another of 70 g., and, to the eye, they have much the same appearance. Tested by the apparatus of the above investigators, the greater hardness of the latter is made apparent.

Although it seems established that certain muscles are able to hold up, by means of the "catch" mechanism, a weight for a considerable time without appreciable consumption of energy, the work of Cohnheim (1912, 3) on *Sipunculus*, and of Cohnheim and von Uexküll on the leech (1912), showed that, when loaded, the energy consumption is greater in these animals than when unloaded. But it is plain that it is difficult to be certain that there is no reflex effect on other muscles or organs, on account of the abnormal conditions present.

An appropriate form of apparatus for the investigation of the phenomena of tonus, especially in the snail, is described by Jordan (1908, 1912).

Of course, the comparison of the mechanism in question to that of a catch or ratchet is only intended to assist the reader in grasping the mechanical conditions present, which are similar in both cases. As to the actual process itself, hypothetical suggestions only can be made in the present state of knowledge. The state of tension into which a skeletal muscle of the vertebrate is put by stimulation passes off automatically when the stimulus is removed. Whatever may be the cause of this increased tension, whether the setting free of some

substance which increases surface energy or osmotic energy, it disappears again spontaneously, under the usual conditions. The permanent tonic state of the smooth muscle, which we have been discussing, might be explained by supposing that the internal changes in the muscle cell, which result in the increase of tension, are prevented from disappearing. The mechanism which is responsible for the bringing about of this temporary irreversibility of the contractile process, may be put into action, or inhibited, by the intervention of special nerve fibres. The state of tension into which excitation puts the muscle thus remains until the external inhibitory influence comes into play and sets going the opposite process associated with relaxation, in which the products of the contractile process disappear.

We have, in fact, indications of something of this sort in the "contracture," associated with fatigue, in the skeletal muscle, as well as in the action of veratrine (page 417) and of certain electrolytes (Mines, 1912). Moreover, we shall see presently that the tonic contraction of decerebrate rigidity presents some peculiarities of this nature, when compared with ordinary reflex or voluntary tetanus.

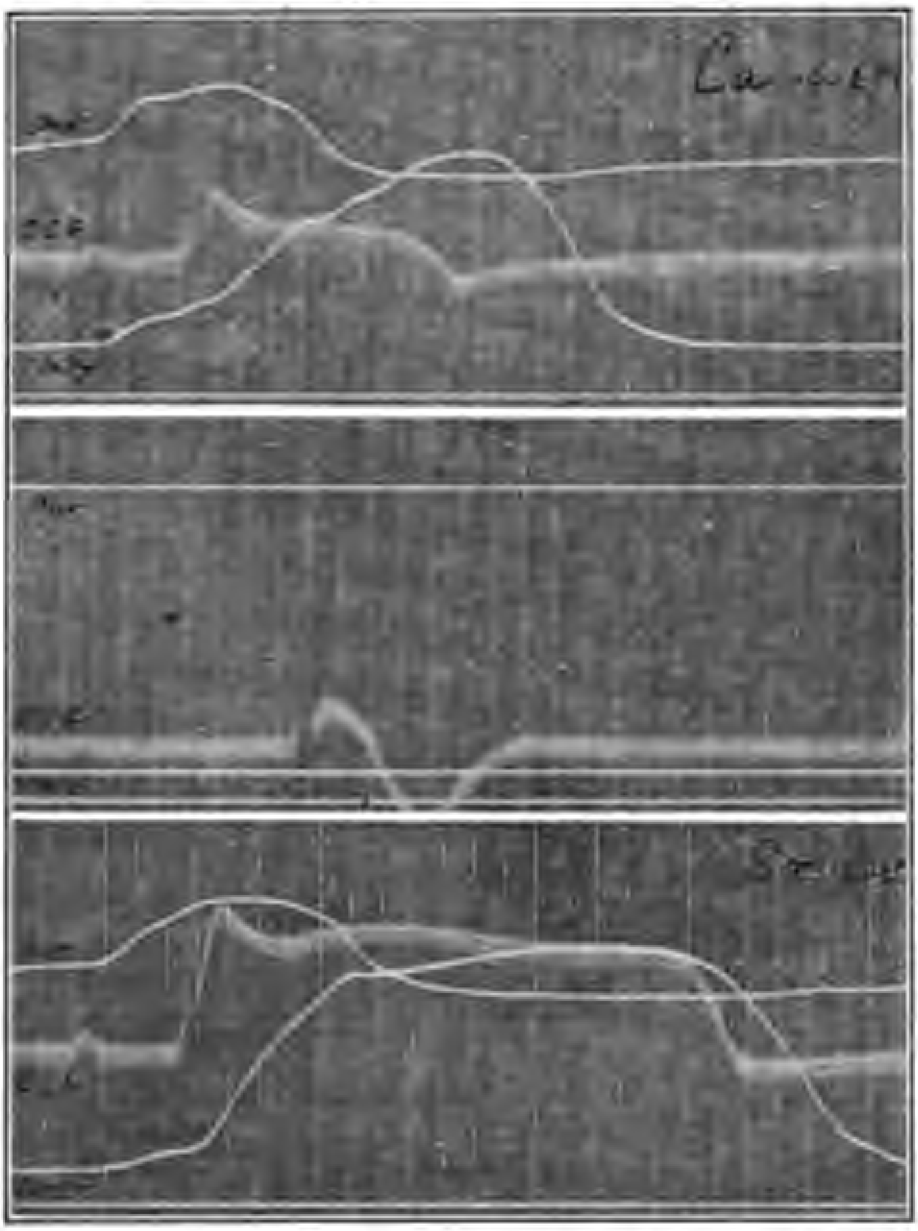


FIG. 172. ELECTRICAL CHANGE WITHOUT CONTRACTION.—The uppermost curve in each of the three tracings is the movement of a lever connected to the auricles of the frog's heart. The middle one is the record of the string galvanometer. The lowest one, the beat of the ventricle. The upper tracing is a normal one, the perfusion fluid containing calcium. The middle tracing shows the effect of omitting calcium. The electrical change persists, but there is no change of form of the muscle. The lowest tracing was obtained after adding strontium in amount equivalent to the calcium of the upper tracing. The beats return, as Ringer showed, but are considerably increased in duration. Note, especially in the last curve, that the electrical change begins and ends rather earlier than the mechanical one, but that the total duration is the same.

(Mines, 1913, 3, p. 230.)

If we suppose the actual state of contraction of the muscle, as well as the excitatory process which precedes the contraction, to be associated with some degree of electrical negativity, and this seems to be the case, at all events, in the heart (see Figs. 172 and 173) and in the voluntary muscle under veratrine (de Boer, 1913, 2), then the observations of W. F. Ewald (1910) are to the point here. Examining the adductor muscle of *Anodonta*, he found two states of electrical negativity, one, the "twitch" current, which appears at the beginning of a reflex closing of the shell, probably the contraction of the motor muscle, and another one following this, a slow one, and apparently proceeding *pari passu* with the degree of tonus of the catch muscle. This latter is, according to the investigator, steady

and not of a discontinuous nature, in fact it seems like that of the mechanical state of the muscle. One would be inclined to associate it with the persistence of the state of tensile stress, which, owing to the existence of the mechanism preventing its disappearance, remains in the state to which it was brought by the stimulus.

If the electrical response is due to the disappearance of the polarised state of some membrane, owing to its becoming completely permeable, it might be supposed that the mechanism consists in some method of maintaining the state of permeability.

THE AURICLE OF THE TORTOISE

The auricle of the tortoise, as shown by Fano (1887), undergoes slow rhythmic changes in tonus, on which the ordinary beats are superposed. Rosenzweig (1903) showed that these tonic contractions are due to the presence of non-striated muscle fibres. Fano and Fayod (1888) had shown that the vagus nerve increases these tonic changes, an action opposite to that which it exerts on the auricular muscle proper. Bottazzi (1900) and Oinuma (1910) confirmed this result, and showed further that the sympathetic nerve inhibits the tonic changes.

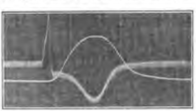


FIG. 173. RELATIVE DURATION OF THE MECHANICAL AND ELECTRICAL CHANGES IN THE AURICLE OF THE TORTOISE.

Upper curve—electrogram.
Lower curve—mechanical response.

(Mines, 1913, 2, p. 333.)

Gaskell (1916, p. 82) points out that the simplest explanation of these results is that the axons of each nerve divide into two fibres, one excitatory, the other inhibitory. Some muscle related to those of the alimentary canal must have become included in the course of the evolution of the heart.

TONUS IN SKELETAL MUSCLE

The tonic state into which certain skeletal muscles, most markedly the extensors of the limbs, fall after separation of the centres below the corpora quadrigemina from the higher parts of the brain, has been described above (page 417). Sherrington (1909, 3) has discovered that this condition shows several remarkable properties, which remind one in many ways of those of the smooth muscles just described, the difference being that, in the skeletal muscle, the phenomena are due to the intervention of the central nervous system. These phenomena are of such a nature as to have led their discoverer to call the state "*Plastic Tonus.*"

Suppose that we start with a limb in the rigid state, with extensors contracted, grasp the fore limb and attempt to flex it. After a certain latent time, the resistance to movement will be felt to relax and the limb can be flexed to any point desired. The striking thing about this degree of flexion is that the limb *remains fixed* in the position to which it was moved. Again, suppose that the tonic rigidity has relaxed spontaneously, as usually happens, and that we place the limb in various degrees of extension, again it remains where it was placed. These phenomena are due to reflexes from the proprio-ceptors in the muscles themselves, since they disappear after section of the dorsal roots containing the afferent fibres from these receptors.

The existence of this tonic reflex from the muscle itself can be shown by stimulating the *uncut* nerve to it at a rate of about four to five stimuli per second. A continuous steady contraction curve is traced; whereas, if the nerve be cut and its peripheral end stimulated in the same way, a series of separated twitches is traced. Similar results can be obtained by causing reflex contractions in the normal and in the "de-afferented" muscle. The former are of a tonic nature even

when the stimuli are separated by intervals, the latter are clonic and consist of a short response to each stimulus.

Since the leg is maintained in a different position, even when it is loaded, it is clear that a particular length of muscle may be in equilibrium with different loads; in other words, the same length of fibre may have different tensions, just as we saw in the case of the involuntary muscle.

Another point of interest is that the reflex contractions of the de-afferented muscle are fatigued sooner than those of the muscle in its normal state.

The mechanism must consist in each particular length of the muscle being able, in some way, to stimulate the receptors of this muscle in such a manner as to maintain the degree of contraction at this level.

We must next consider certain experimental facts which show that this reflex tonic contraction is a different thing from a steady contraction of the same height, produced by the application of repeated induction shocks to the cut nerve at a sufficient rate to give a fused curve, or from the ordinary spinal reflex described in Chapter XVI.

Inhibition of Tonus.—It will be remembered that Sherrington showed that, in the case of spinal reflexes, by appropriate relative strength of stimuli applied to different nerves, one excitatory, the other inhibitory, any degree of reflex contraction of the vasto-crureus can be obtained. Curves illustrating this fact are given in Figs. 118, 122, and 123, on pages 410-415 above, and a further one in the upper curve of Fig. 174. But, supposing that, instead of the ordinary reflex contraction, which can be reduced to any desired extent by different strengths of the stimulus to the inhibitory nerve, we take the tonic contraction of decerebrate rigidity and attempt to reduce it to different degrees by varying the strength of the stimulus of the same inhibitory nerve. It was shown by Sherrington (1909, 2, pp. 256 and 257) that no algebraical summation is possible; any strength of stimulus which has any action at all produces a gradual fall in the height of the tonus, which fall continues until complete relaxation results, if the stimulation is continued long enough. In other words, instead of falling rapidly to a certain point and remaining there, the tonus completely disappears. The only difference between the effect of strong and weak stimuli is the *rate* of fall, as is seen in the lower curves of Fig. 174. One is again reminded of the removal of the "catch" in the inhibition of the adductor muscle of the scallop, although the mechanism is peripheral there, central here.

Fröhlich and Meyer (1912) again, have noticed phenomena with tetanus toxin which lead them to regard the relaxation of mammalian muscle as being, in some way, directly under the control of the central nervous system. If a particular segment of the spinal cord is poisoned with this toxin, the muscles supplied by this segment enter gradually into a state of shortening. In this state, the metabolism of the muscle appears to be abnormally small; glycogen accumulates in it. It gives no muscle sound and no vibration on the string galvanometer. If, however, the muscle is passively stretched by pulling upon it, the muscle sound is heard and the galvanometer shows the characteristic vibratory current of action of voluntary tetanus. On the view suggested above, it might be supposed that the process (production of lactic acid) which is responsible for the increase of tension has become, so to speak, permanent; hence the state of surface tension does not disappear spontaneously, owing to the removal of the acid under the influence of oxygen, but requires some nervous influence to set the necessary mechanism into play. The phenomenon differs, however, from those of involuntary muscle in that, in the cases under discussion here, it is of central origin, as remarked in the preceding paragraph.

Metabolism.—In the experiments of Fröhlich and Meyer, as given above, it was noted that the metabolism was unusually low. Now Roaf (1912) has described experiments in which he found that the carbon dioxide output and also the oxygen intake were no greater in the state of decerebrate rigidity than in a subsequent period in which the contraction was abolished by the use of curare.

It should be stated that Lovatt Evans found the metabolism to be less under curare than in decerebrate rigidity. Of course, care was taken in both sets of experiments to prevent fall

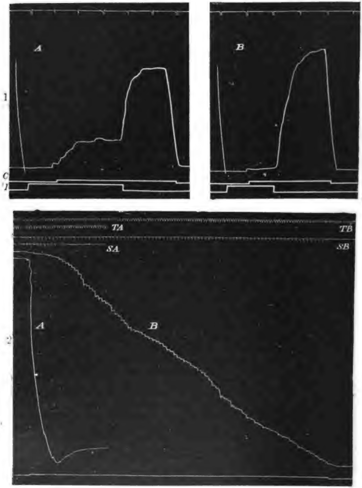


FIG. 174. INHIBITION OF TONIC CONTRACTION.

1. *A*, Reflex contraction of vasto-crureus muscle in decapitated cat. At the rise of the signal *I*, the central end of an inhibitory nerve (ipselateral peroneal) was stimulated. Since the muscle was free from tonus, no apparent result was obtained. At the rise of the signal *C*, an excitatory nerve (contralateral peroneal) was stimulated—a certain degree of contraction. When the inhibitory stimulus was removed, there was a much greater contraction, showing that the previous condition was one of balance. Time in seconds above.

1. *B*, A similar experiment, with relatively greater strength of inhibitory stimulus. The grade of contraction is less than in 1, *A*.

Note the continued steady position of the muscle in the different degrees of relaxation.

2. Vasto-crureus in decerebrate rigidity. Reflex inhibition produced by stimulation of the ipselateral peroneal nerve, with slowly repeated break induction shocks shown by signal lines *SA* to *SB*. Time in fifths of seconds by signal *TA—TB*.

In experiment *A*, the strength of the stimulus was greater (100 Kronecker units) than in ***B*** (20 Kronecker units). In both, the relaxation proceeds to the full resting length of the muscle; but, in *A*, it is rapid and attained after nine shocks, while in *B* it is reached slowly in eighty-six shocks of the weaker stimulus. The signal line at the foot of the figure marks the duration of the stimulation in *B*.

Note that no permanent intermediate state, due to balance of degrees of excitation and inhibition, is obtained in this state of decerebrate tonus.

(Sherrington, 1909, 2, pp. 256-257.)

in the temperature of the preparation. In any case, it seems fairly certain that much less metabolism is associated with the tonic form of contraction. This result again is to be compared with the experiments of Parnas and of Bethe given above.

The reader may also be reminded of the inefficiency of the sartorius muscle of the frog when maintaining a weight by stimulation of its nerve with induction shocks. This result led A. V. Hill (1913, 4, p. 319) to suppose that there must be a more efficient mechanism for the purpose in the normal organism. It is suggestive that Hill found, in the same series of experiments (p. 317), that the amount of heat produced per unit of tension developed, is independent of the frequency of the stimuli, provided that the latter are sufficiently rapid to cause complete fusion of twitches.

Pembrey (1903) noticed that the *panniculus carnosus* of the hedgehog, which keeps the animal rolled up into a ball, was in a state of tonic contraction during hibernation, a fact which adds confirmatory evidence to the view of Roaf that decerebrate rigidity is not associated with any considerable increase in metabolism.

Heat Production.—I have myself made some experiments (1912, 3) on the heat production in muscles in decerebrate rigidity. Although the work is not yet complete, I found that there is a certain amount of heat produced, in magnitude varying with the degree of tonic contraction, although it is undoubtedly very much less than that produced in an artificial tetanus of a similar height.

Electrical Change.—According to Buytendyk (1912), the electrical change in decerebrate rigidity, as shown by the string galvanometer, is discontinuous; a fact which leads him to regard it as a periodic discharge from nerve centres, similar to tetanus. Hofmann (1913) finds a similar oscillatory discharge in the normal tonus of the eye muscles of the rabbit. We are not compelled, however, to consider this to be the same thing as ordinary tetanus; the putting in action or removal of the "catch" mechanism might take place in a series of discharges. Sherrington's inhibition experiments show that the relaxation is not instantaneous. The amplitude of the electrical waves is less than in ordinary reflex tetanus. But it is not possible to lay much stress on this fact, since it might be caused by the phases of contraction not being synchronous in all fibres. Buytendyk, however, points out that the oscillations in his curves are very regular, which seems to indicate synchronous state of contraction in all fibres.

Production of Creatine.—Pekelharing and van Hoogenhuyze (1910) found an excess of creatine in invertebrate muscle in tonus, and Pekelharing (1911) found creatinine in the urine of men after prolonged voluntary tonic contraction, but not after walking. The results of Cathcart and Leathes on uric acid have been mentioned previously (page 289). Leathes and Orr (1912), further, repeated Pekelharing's experiment and found both uric acid and creatinine increased.

Relation to Labyrinth.—Ewald (1894) pointed out the important relation of the labyrinth to the maintenance of tone in the muscles generally and the loss of tone resulting from destruction of the semicircular canals. More detailed investigations were made by Magnus and De Kleijn (1912) by the use of a method devised by the latter. It was found that the tonic contraction of the limb muscles in decerebrate rigidity, especially those of the fore limbs, was greatly influenced by changing the position of the head. Further analysis showed that there are two factors at work, reflexes from receptors in the labyrinth and reflexes from proprio-ceptors of the muscles of the neck. The former are concerned with the relation of the head to space, independently of its relation to the trunk. The latter are concerned merely with the position of the head in relation to the trunk. The labyrinth can be rendered inoperative by the injection into it of a 20 per cent. solution of cocaine, according to the method of De Kleijn (1912). The neck effect can be excluded by making the neck immobile on the body by encasing it in plaster of Paris. It was found that the labyrinth receptors are not affected, as regards their influence on tonus, by change of position in a horizontal plane, but changes in the vertical plane have great effect on the tonus of the limbs. When the head is in such a position that the vertex is upwards and the nose at an angle of about 45° looking downwards, extensor tonus is minimal; with the vertex downwards, and the nose at 45° upwards, that is, on rotation of 180°, tonus is maximal. It was noticed that, along with contraction of the extensors, there was inhibition of the flexors. Moreover, in this connection it is interesting to note that Magnus and Wolf (1913) subsequently found that

strychnine does not reverse the inhibitory phase of this reflex, at all events in any possible dose.

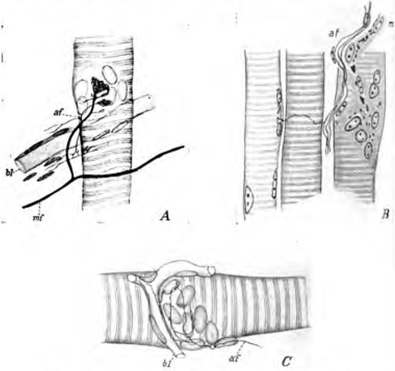


FIG. 175. ACCESSORY (SYMPATHETIC) NERVE-ENDINGS IN VOLUNTARY MUSCLE.

A, From intercostal muscle of young rabbit. Magnified 1,700 times.
bl, Blood vessel, partially covered by the muscle fibre.
af, Accessory nerve fibre, which is seen to be connected with the perivascular (sympathetic) nerve plexus. This fibre ends in a special structure, independent of that of the motor fibre.
mf, Motor fibre, with branch, ending in ordinary end-plate.

(Boeke, 1911, Fig. 49.)

B, From a section of the superior oblique muscle of the cat's eye. After section of the trochlearis nerve and degeneration of the motor fibres.
m, Degenerated motor-nerve fibres with remains of end-plate.
af, Accessory fibres intact. The hypolemmal ending of one is seen in profile.

(Boeke, 1913, Fig. 10, p. 353.)

C, Ending of fine non-medullated fibres, *af*, on muscle fibre of the rectus superior oculi of the cat. These endings lie in a granular, nucleated protrusion of sarcoplasm. The nerve fibres themselves have nuclei here and there.
bl, A blood vessel, upon which a fine branch of the accessory fibre, *af*, is distributed.
Magnified 1,800 times.
Silver impregnation of sections from material fixed in formaldehyde; sometimes treated with gold afterwards

(Boeke, 1911, Fig. 53.)

The neck reflexes were also found to follow definite laws, for which the original paper of Magnus and De Kleijn must be consulted. By combination of the two factors, all the complex phenomena of the decerebrate reflex tonus could be explained.

The fact that the labyrinthine reflexes are only to be obtained by changes of position in relation to gravity, and the fact that they are permanent as long as

the new position lasts, indicate that they proceed from statolith organs, rather than from the semicircular canals themselves.

The change of tonus, as just remarked, is a permanent one, as long as the new position of the head is maintained.

In the experiments of Magnus and Wolf (1913), the preparation was so made that the changes in length of the isolated triceps or vasto-crureus could be traced on smoked paper. The above results were confirmed, and it was shown very clearly how, in this tonic state, a muscle can have different lengths under the same load.

Relation to Sympathetic Nerves.—Certain facts have been brought forward recently which appear to indicate that the tonic state of skeletal muscle may have something to do with sympathetic innervation of this kind of muscle. But caution must be exercised until further work. Perroncito (1902) and Boeke (1911) described accessory nerve endings in various voluntary muscles, which appear to be of sympathetic origin (see Fig. 175). In preparations A and C of Fig. 175, it will be seen that there is continuity of the fibre going to the accessory ending with the plexus around the small blood vessels. In a later paper (1913), Boeke shows that the accessory endings do not degenerate on section of the motor nerves to the eye muscles, whereas the motor endings do (see Fig. 175, B). De Boer (1913, 1) finds that the normal tonus of the hind limbs of the frog and of the cat disappears when the rami communicantes of the sympathetic ganglia are cut.

If this be so, it seems that stimulation of the sympathetic should produce tonic contraction of the muscles. In some experiments in which I stimulated the sympathetic of the frog for another purpose, I did not observe any effect of this kind, but experiments should be made, both on the frog and on the cat, for the special purpose.

In a further paper, De Boer (1914, 2) finds that rigor mortis is more marked on the normal side than on that in which the sympathetic rami have been cut. This result suggests that the tonic state is associated with difficulty of removal of the products of metabolism. The same investigator (1913, 2, and 1914, 1) brings the prolonged contraction, caused by a single induction shock to a veratrinised muscle, into connection with the normal tonic state. The electrical state corresponding to this veratrine contraction is that of a prolonged steady negativity of the longitudinal surface to the tendon, as if a continuation of the normal brief state of negativity. De Boer thinks that the prolonged contraction is due to the "sarcoplasm," as suggested by Bottazzi, and similar to that of smooth muscle. A difficulty in this interpretation of the veratrine curve is that a stimulus to the spinal cord, after section of the sympathetic rami, produces a prolonged contraction in a veratrinised frog. De Boer suggests that the accessory endings of Boeke might be directly stimulated by the initial ordinary twitch.

According to this view, normal tonus is also associated with a slow consumption of protein material in the sarcoplasm under the influence of the sympathetic system. It is true that the appearance of creatinine and uric acid in the urine, referred to above (page 289), would be thus accounted for, but further evidence is required.

It has recently been shown by Kure, Hiramatsu, and Naito (1914) that the diaphragm is kept in a state of tonus by impulses from the sympathetic system, conveyed by the splanchnic nerves. When these nerves are cut, the diaphragm is drawn up into the chest by the negative pressure in the pleural cavity.

Certain effects of *adrenaline* on skeletal muscle, which have been described, are of interest in the present connection. As we shall see in Chapter XXII., this product of the activity of the suprarenal glands has the special property of exciting the nerve endings of the sympathetic system and producing the effect due to stimulation of sympathetic nerves themselves. Has it then any effect on skeletal muscle? Oliver and Schäfer (1895, p. 263) found that the twitch of the voluntary muscles in the frog and in the dog was considerably prolonged after an injection of suprarenal extract, an effect similar to that of veratrine. But if the sympathetic endings of Boeke were stimulated by the drug, one would expect that a shortening of the muscle would take place without the application of a stimulus to the nerve. Cannon and Nice (1913) have described experiments in which injection of adrenaline enabled a muscle *in situ* in an animal to continue contracting longer, without fatigue, than in the absence of the drug. This effect is

regarded, no doubt correctly, as being for the most part due to increased blood supply from the rise of arterial pressure caused by the adrenaline. At the same time, the investigators are inclined to think that there is also a direct effect, since that on the muscle lasts longer than the rise of blood pressure. But might it not be the result of the previous increase of blood supply? More convincing is the experiment in which the blood pressure in the limb under experiment was prevented from rising by compression of the artery. The effect was still present. According to Panella (1907) and Gruber (1914), adrenaline is an antagonist to curare, hence it must act on some receptive substance in the muscle.

Kuno (1915) failed to obtain any evidence that adrenaline has an action on the voluntary muscle of the frog. But this result does not definitely exclude sympathetic innervation, since adrenaline does not excite the sweat glands, although they are supplied by the sympathetic. Cannon and Cattell (1916, p. 75), moreover, saw galvanometric deflections from voluntary muscle when acted upon by rather large doses of adrenaline, of the sign indicating excitation.

From the morphological aspect there appears to be some difficulty with respect to the sympathetic innervation of voluntary muscle. In certain cases, as we have seen, the two kinds of function are performed by two separate kinds of muscle fibres, as in the auricle of the tortoise, or by separate muscles, as in the mollusc. But in other cases, as in the vertebrate bladder or in that of voluntary muscles, the same fibres undertake both functions, so far as can be made out. Whether the one is performed by the sarcoplasm, as held by Bottazzi (1897), and the other by the fibrils, remains undecided. It is not easy to understand how two fibres of different function and different innervation could coalesce with retention by the combined cell of both kinds of innervation, although the case of the salivary glands suggests the possibility.

Posture.—It is rightly insisted on by Sherrington (1915, p. 224) that the name "tonus" for the phenomena dealt with in the present chapter is an inappropriate one. The existence of tension is implied in it, whereas, as we have seen, the varying lengths which a muscle can be made to assume, in particular conditions, are quite independent of tension. The conception of posture, as illustrated by the muscles in the decerebrate animal, may, with great advantage, be transferred to the smooth muscle of hollow viscera and blood vessels. Just as it is possible to hold in the hand either a large or a small ball, exerting upon each the same slight pressure, so the bladder is able to hold a large or small quantity of fluid with the same internal pressure, or even "a large volume with a lighter pressure than it may exert at another time on a smaller volume." We speak of the hand as "adopting a posture suited to the volume of the object it grasps" and, correspondingly, "The bladder assumes postures suited to the volume of its contents."

Contractures. — From experience derived from the treatment of injuries to nerves and of fractures of bones, it appears that the fixing of a muscle in such a position that it is stretched is liable to lead to a state of shortening. It is probable that this is partially due to reflexes from proprioceptors, although growth of fibrous tissue may occur. The beneficial results ascribed by Leriche and Heitz (1917) to vaso-dilatation when they divide the peri-arterial sympathetic nerves, or resect a piece of artery, in cases of spasmodic contraction, may be connected with the abolition of reflexes to the sympathetic endings in voluntary muscle.

Action of Drugs.—That a state of muscle similar to that of postural activity can be induced by certain drugs has been referred to incidentally in connection with veratrine. The phenomena associated with the action of this drug suggest the participation of two distinct mechanisms in voluntary muscle. There are other drugs that produce what is called tonic contraction in both voluntary and smooth muscle, as strophanthin on the heart, or barium on voluntary muscle. Thus, the mechanism can be called into play otherwise than through the action of nerves.

Theory.—The author is of opinion that the view most in accordance with all facts is that, as already indicated, the tonic state is due to permanence of the process which has given rise to the ordinary form of contraction. Thus it is a function of the fibrillæ in voluntary muscle, and may possibly be brought about by sympathetic innervation. But it is clear that further investigation is needed.

AUTOMATIC ACTIVITY OF NERVE CENTRES

Certain nerve centres, such as the respiratory and vasomotor centres, are in constant activity. The question arises as to whether the state of excitation in such cases is actually automatic, or whether it is kept up by afferent impulses from the periphery. There can be no doubt that such impulses are able to modify the state of the centres, and also that chemical substances, such as hydrogen ions, in the blood, are able to set up a state of excitation in nerve centres. The heat regulating centre is itself sensitive to heat and cold. Thus, afferent nerve impulses are not always necessary. It is not easy to decide whether there is an automatic state of excitation apart from stimulating substances in the blood, although it seems possible. There are, obviously, many centres which must not be active, except for special purposes; such are those of the voluntary muscles. Even here, however, the question arises as to whether the inactivity of centres not in use may be due to inhibition (see the experiments of Pavlov on conditioned reflexes, pp. 503-506).

SUMMARY

Involuntary, smooth muscle, in its various situations, is capable of remaining in a state of moderate contraction, or tonus, independent of any influence from nervous centres.

Certain muscular systems in invertebrates, such as that which closes the shell in bivalve molluscs, and many others, are able to maintain a shortened state against a heavy load for a long time without any evidence of fatigue.

This state of "fixation" may occur at any length of the muscle fibres, and with any tension.

A similar condition is met with in the urinary bladder of mammals, and probably in the muscular coat of the arterioles, as well as in other situations.

It may be compared to the putting into action of a "catch" or ratchet mechanism.

To put the "catch" into action, or to remove it, requires the intervention of nerve impulses from centres; these impulses would be excitatory in the first case, inhibitory in the second. They are conveyed by distinct nerve tracts.

The actual mechanism may possibly consist in the prevention of the spontaneous disappearance of the products (lactic acid) caused by stimulation of the muscle system, by which its potential energy is converted into that of tension.

The "plastic tonus" of skeletal muscle, described by Sherrington in the decerebrate animal, appears to be of similar nature, although the mechanism is in the nerve centres in this case, instead of being peripheral.

In this state the vasto-crureus, for example, may be maintained at different lengths with the same load, or at the same length with different loads. Thus the fibres may have a different tension with the same length.

The phenomenon is a reflex from proprioceptors in the muscle itself.

Decerebrate tonus behaves towards inhibitory stimuli in a manner different from that shown by ordinary reflexes.

There is some evidence to show that this decerebrate rigidity is not accompanied with increase of metabolism, or with comparatively little.

It is affected reflexly from muscle receptors of the neck and from the labyrinth.

Certain facts described in the text indicate a relationship of tonus in skeletal muscles to a sympathetic innervation of these muscles.

The phenomena as a whole are best described as adjustments of posture, since the name tonus suggests tensile strain.

The question as to the automatic activity of nerve centres is discussed briefly in the text.

LITERATURE

General.
Sherrington (1915).

Mechanism in Invertebrates.
Von Uexküll (1912).

Plastic Tonus.
Sherrington (1909, 3).

Relation to Position of Head.
Magnus and De Kleijn (1912).

CHAPTER XIX

THE ACTION OF LIGHT

SINCE the whole existence of living organisms on the earth depends on the receipt of radiant energy from the sun, it is unnecessary to point out the importance of the study of the way in which this energy is made use of. If it were merely converted into that form of energy in material bodies which we know as heat, it would be a very wasteful process, as our study of energetics has taught us. Much of the free energy would be thereby lost in the process of conversion to other forms. A considerable part of the sun's energy is, of course, used up in warming objects on the earth, but the study of the chemical reactions brought about by the action of light is of greater importance, although of some difficulty.

The amount of energy actually received from the sun may be somewhat realised by the following data: Suppose that the atmosphere were absent and the sun in the zenith, then each square centimetre receives per minute 1·955 small calories, expressed in heat units. The presence of the atmosphere, which absorbs a part of the radiations, reduces the value to 1·2 small calories, at the latitude of Cambridge. In other words, the energy received by 1 sq. m. (10,000 sq. cm.) in one minute would suffice to raise the temperature of a kilogram of water by 12°.

The reader will not need to be reminded that the most important of all photo-chemical reactions is that by means of which the chlorophyll system of the green plant stores up light energy and, in the process, restores to the atmosphere the oxygen which has been used up in oxidation by living beings. The stores of energy in coal and petroleum also owe their origin to chlorophyll in past ages. indirectly in the latter case, if we accept its animal origin.

In order that we may be in possession of the means of understanding, as far as is possible at present, the mechanism of the process concerned, we must first enter somewhat fully into the general theory of photo-chemical reactions. In addition to the chlorophyll system, there are other actions of light which are of physiological importance. Such are the retinal process and the action of ultra-violet light. In practical use, the various photographic methods may be mentioned, as well as those of wireless telegraphy, which makes use of waves like those of light, but of very much longer wave length.

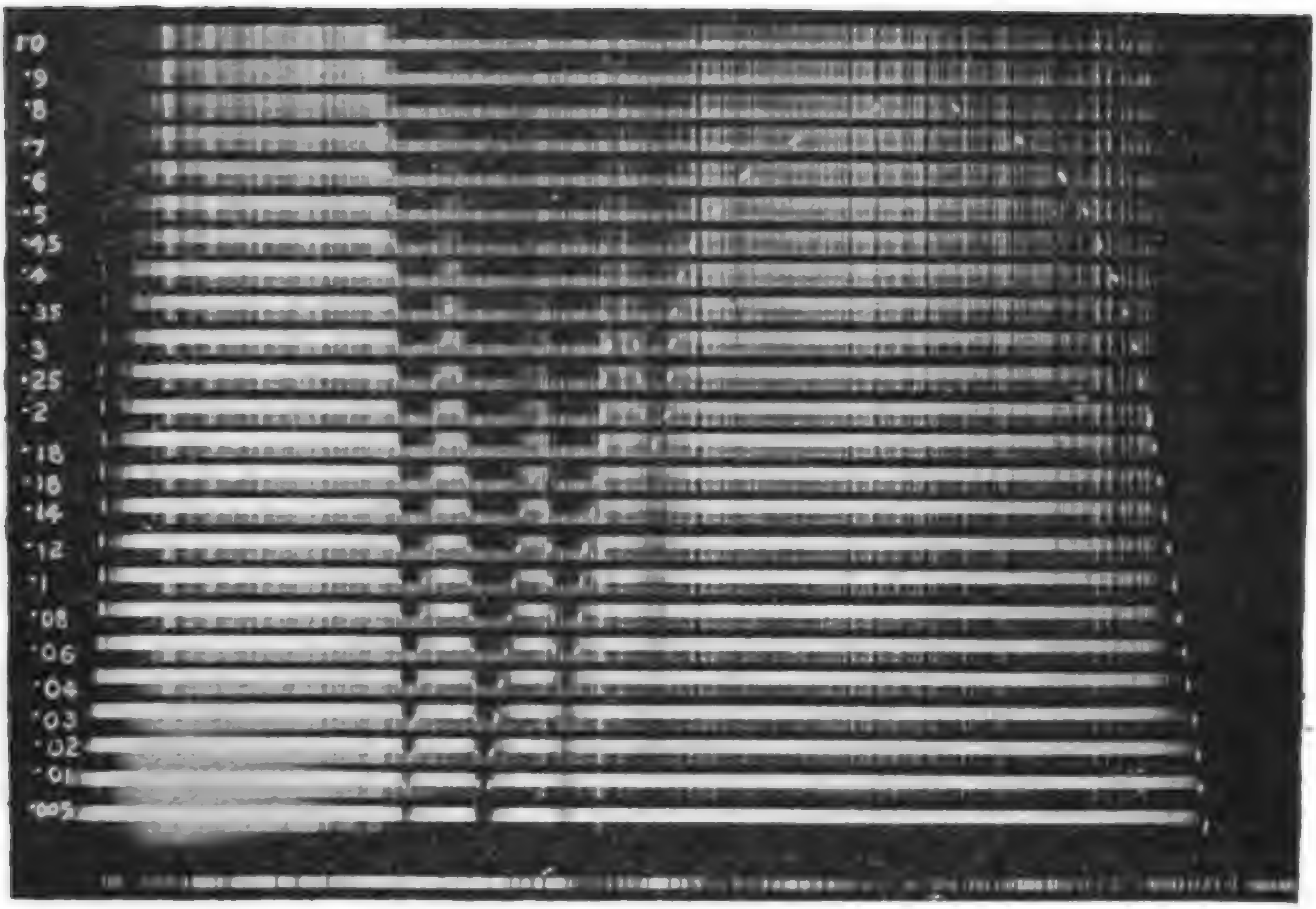
ABSORPTION OF LIGHT

All substances absorb radiant energy to some extent; glass itself absorbs rays of longer wave length than those we know as light, and also those of shorter wave length. The colourless substance, anthracene, absorbs ultra-violet rays, as is shown in the photograph of Fig. 176, and many other instances might be given. In other words, a part of the energy of a beam of light which traverses any substance is removed and held back in the substance. Something must, therefore, happen to the substance; it may be merely warmed, or other forms of energy may make their appearance in it, chemical or electrical change, and so on.

Grotthus's Law.—It seems fairly obvious to us at the present time that no effect can be produced by light unless it is absorbed. We shall see presently also that some light energy must be used up to start any photo-chemical change, even when the reaction afterwards proceeds with evolution of energy. The law that light must be absorbed in order to produce an effect was first clearly enunciated by Grotthus (1819, p. 101) and, independently, at a later date, by Draper (1841). It is frequently known as Draper's law.

Grotthus found, for example, that ferric thiocyanate, which is red, is decolorised by

APERTURE OF *A* ADJUSTABLE SECTOR.



B

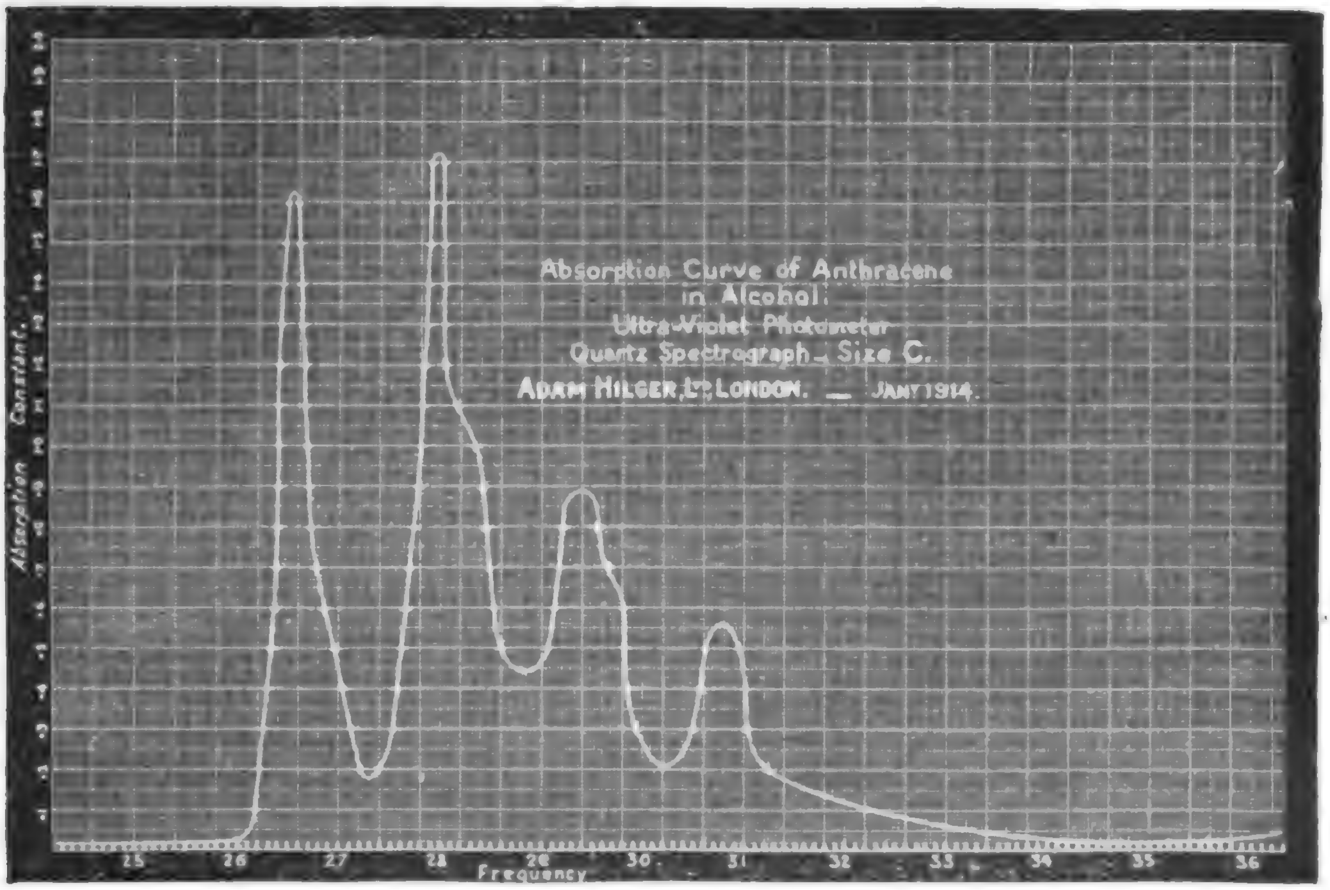


FIG. 176. ULTRA-VIOLET ABSORPTION BY ANTHRACENE.

A, Series of photographs of ultra-violet absorption spectra of anthracene. Below each one there is a normal spectrum. The relative intensities of the two spectra are varied in known ratio by means of rotating adjustable sectors. At a certain wave length in each pair, marked with a white spot, the intensities of the light are equal.

B, Absorption curve drawn from the above photograph.

Ordinates—amount of absorption corresponding to the frequency of vibration of light indicated by the abscissæ.

(See Catalogue of Messrs Adam Hilger, 1914.)

green light, yellow gold chloride by blue light, blue starch iodide by yellow light. Each is attacked by light of the colour complementary to its own colour, that is, by the light which it absorbs.

The Laws of Lambert and of Beer.—In order to be able to compare the amount of light absorbed by one substance or solution with that absorbed by another, it is necessary to take some standard of measurement. Bunsen and Roscoe (1855-1859) introduced the *extinction coefficient* for this purpose. Their definition of it will be found on p. 6 of the reprint in Ostwald's "Klassiker," No. 38.

When light of a particular wave length is absorbed by any substance, it is clear that the intensity of the light issuing from it is less than that which enters it, and that there must be some particular thickness of it which reduces the intensity of the light to one-tenth of the value it had on entering. In order that the numbers, characteristic of different substances, should rise or fall in the same direction as the absorbing power of the substance or solution, Bunsen and Roscoe defined the extinction coefficient as being the reciprocal of the depth of the solution required to reduce the intensity of light of a given wave length to one-tenth of that which it had on entering. It is plain that the *greater* the absorbing power, the *less* the depth required; hence the advantage of the inverse value, the extinction coefficient being directly proportional to the absorbing power.

The symbol ϵ is generally used for the extinction coefficient, so that if d is the depth of solution required to reduce light of a given wave length to one-tenth of its value, then the extinction coefficient for this wave length is

$$\epsilon = \frac{1}{d}$$

Now in practice it is the intensity of the issuing light that is measured, and it is more convenient to use a constant thickness of solution, and to measure the intensity of the light that has passed through this, than to vary the thickness of the absorbing layer. It is therefore necessary to know the laws which express the relation of the one to the other.

We have already seen (page 35) that the relation is a logarithmic one, and it is known as Lambert's law when applied to the case of a pure substance, solid or liquid. Beer showed that the same law applies to solutions. Let us call the original intensity of the light I, and that after passing through a layer d, I′. Then, by the definition of the extinction coefficient, $I' = I \times \frac{1}{10}$. After passing through a second layer, $I' = \left(I \times \frac{1}{10}\right) \times \frac{1}{10} = I \times \frac{1}{10^2}$ and after x such layers $I' = \frac{I}{10^x}$. In general, if light of unit intensity is reduced to $\frac{1}{n}$th by a certain thickness of solution, after passing through x times this thickness, its value will be $\frac{1}{n^x}$. In order to get rid of the exponent we take logarithms, thus:—

$$\log I' = -x \log n. \qquad (1)$$

When $x = d$, $I' = \frac{1}{10}$, therefore

$$\log\left(\frac{1}{10}\right) = -d \log n = -1 \text{ or } d \log n = 1.$$

$$\epsilon \text{ is} = \frac{1}{d} \text{ and is therefore} = \log n.$$

$$\text{From equation (1), } \log n = -\frac{\log I'}{x}.$$

Hence, $\epsilon = -\frac{\log I'}{x}$ and, knowing the ratio of the light transmitted to that entering a solution of depth x, we can calculate the extinction coefficient.

For convenience, x is taken of one centimetre depth, so that $\epsilon = -\log I'$, that is, *the negative logarithm of the unabsorbed light.*

For example, suppose the intensity of light of the wave length of the D line is reduced to

two-thirds of its intensity by passing through a stratum of a particular solution of one centimetre thickness. Then,

$$\epsilon = -\log\frac{2}{3} = \log 3 - \log 2 = 0{\cdot}176091.$$

By Beer's law, the absorption of light by solutions is directly proportional to the concentration; so that, if we know the extinction coefficient for a known concentration, we can estimate an unknown concentration by measuring its extinction coefficient. Hence the practical value of the form given to the extinction coefficient. Thus:—

c and c' being the respective concentrations, and

ϵ and ϵ' the respective extinction coefficients,

$$c : c' = \epsilon : \epsilon',$$

$$\text{or } c' = c\frac{\epsilon'}{\epsilon}.$$

Such measurements have played a large part in the investigation of the blood pigments.

Resonance.—Light consists of a series of periodic electro-magnetic disturbances of various periods of vibration, or wave length. We have seen (page 88) that when a system, such as a pendulum, has the same period of vibration as that of a series of minute impulses delivered to it, the system is set into vigorous movement by the heaping up of the effect of a number of small impulses. It is, in fact, a means of accumulating energy. Consider now the effect of a set of various wave lengths, such as we find in the sun's light, on a chemical molecule, which has itself a definite rate of vibration. Some of the rates of vibration of the different light waves will almost certainly coincide with that of the molecules of the absorbing substance and will therefore set these into resonant vibration, which may reach an amplitude great enough to bring about chemical change. At the same time, those rays of the vibration period in question will be absorbed and, if situated in the visible part of the spectrum, there will be an *absorption band* seen by the eye. If in the ultra-violet, as in the case of many colourless organic compounds, the band, although invisible, may be photographed.

Spectrophotometry.—Observations by the spectroscope give us information of the position of absorption bands, but we often require measurements of the degree of absorption by different substances in various regions of the spectrum. This is done by the method of spectrophotometry, based on the laws of Lambert and Beer.

In practice it consists in the comparison of the intensity of the light of a particular wave length, which has passed through a known thickness of the solution investigated, with light of the same wave length which can be diminished in intensity in a known degree.

The practical methods of doing this and of calculating the extinction coefficients will be found in Gamgee's article (1898, pp. 213-225). The price lists issued by Messrs Adam Hilger are instructive, especially with regard to the beautiful instruments made by them for the registration, photographic or otherwise, of spectrophotometric measurements. Fig. 176 (page 549) is a copy of the curve of the ultra-violet absorption of anthracene as obtained by one of these instruments. The paper by Eckert and Pummerer (1914) deals with photographic registration. Hartridge (1915) has described an improved spectro-photometer.

GENERAL THEORY OF PHOTO-CHEMICAL ACTION

We may take it then that light of some particular wave length is absorbed, and that it sets into resonant vibration the molecules of the absorbing substance, if any of the vibration periods of the light waves coincide with those of the latter. What is the further course of events? We know that, in many cases, chemical reaction follows.

Let us see first what are the general phenomena with which we have to deal. The article by Luther (1908) may be referred to for more details of the general theory than can be given here, and the monographs by Weigert (1911) and Sheppard (1914) for the whole subject.

In the first place, we find that the rate of the reaction does not follow the simple law of *mass action*. This is due to the fact that it is controlled by the amount of light energy absorbed per unit time and not by the actual number of molecules present. An instructive case is that of the oxidation of quinine by chromic acid in light, as investigated by Luther and Forbes (1909). The order of this reaction depends on the colour of the light; violet light is only slightly absorbed and the reaction is unimolecular, ultra-violet is strongly absorbed and the order is very much lower; since this light is *totally* absorbed, the rate of the reaction is independent of the concentration of the reacting substances.

A curious result of this fact is that the order of the reaction depends on the thickness of the layer of solution through which the light passes. In a thick layer the relative amount of violet light absorbed increases, so that the order of the reaction is higher than in a thin layer, where the violet light is scarcely absorbed at all. We have then a reaction, whose order depends on the shape of the vessel in which it takes place.

In practice, it is found that the majority of reactions brought about by light are of the nature of *oxidations or reductions*, that is, reactions in which changes of valency take place, as we shall see in more detail in the next chapter. But all kinds of reactions are also to be met with.

As the phenomena of *resonance* imply an increase of free energy in the system concerned, it is not unexpected to find that when chemical change takes place it is in the direction such that the resonance is diminished or ceases, according to the second law of energetics.

The mechanism of this resonance process is, according to Luther (1908), essentially as follows, on the basis of the electro-magnetic theory of light, the electronic (atomic) nature of electricity, and the electrical nature of chemical combination. Compounds consist of molecules or atoms smaller than these and between these constituent elements, that is, between their electrons, there is an electric field. The stronger the field the firmer the combination, or the more inactive the compound, and the shorter the period of vibration of the (negative) electron; that is, the further in the ultra-violet the absorption bands lie, the more stable the compound. Conversely, the further the absorption band lies towards the red end, the more sensitive is the compound to light. Thus anthracene, with its bands in the ultra-violet, is less sensitive than chlorophyll, with its band in the red. Researches by Luther and Nikolopoulos (1913) on a series of organic compounds confirm this view.

Further, when the periodic alternating electric field of light acts on the electrons, resonance comes into play, their energy content rises, and would do so indefinitely if it were not changed into heat by some kind of "damping" (possibly due to impacts). In the work of Luther and Nikolopoulos, referred to above, it was found that the steeper and higher the absorption curve, the more sensitive to light. Such a curve, in fact, means a small degree of damping, and great amplitude of vibration of the electrons set in motion by light.

The extent of the loss by damping determines the *efficiency* of the photo-chemical process, which may be very high, as we shall see when discussing the chlorophyll system. The following illustration given by Luther (1908) may perhaps make clear the possibility of a high efficiency. The substance sensitive to light is compared to a reservoir into which air is pumped, the compressed air representing the radiant energy of light. The pressure of the air (*i.e.*, the energy of resonance) inside the reservoir would rise indefinitely except that a hole, D, is provided, through which air can escape, this loss representing the change to heat by damping. Suppose, however, that there is another hole, C, of adjustable aperture, through which air can escape. This represents the change of part of the energy of resonance to chemical work. The pressure will then decrease according to the area of C, and with it, the amount of escape through D (= degradation to heat). If C is made very wide, all the air pumped in escapes through it, and none through D.

Resonance energy thus tends to decrease, either by change of rate of vibration of the resonator, or by increase of damping. Hence, in light of a given frequency of vibration, systems insensitive to it arise from those sensitive to it.

Although light energy cannot act unless absorbed, it does not follow that, when absorbed, *chemical* change always results; for example, acetic anhydride has an absorption band between wave lengths 320 and 240 $\mu\mu$, which is associated with decomposition. But it also absorbs the extreme ultra-violet, apparently by means of its CH_3 group, and resonance of this group does not lead to change.

A fact common to all photo-chemical reactions may be mentioned here, namely, that the action of light is similar to that of a *high temperature*. The dissociation of carbon dioxide and of hydrochloric acid, the conversion of oxygen to ozone, and the polymerisation of anthracene may be referred to. For certain theoretical conclusions, drawn by Warburg from this fact, Weigert's monograph (1911, p. 94) may be consulted.

A theory has been developed by Bodenstein (1913) according to which the first effect of light is to decompose a group into electron and electro-positive remainder. Each of these gives rise subsequently to chemical changes of a particular kind.

PHOTO-CHEMICAL REACTIONS THEMSELVES

When we proceed to examine the various reactions which occur under the influence of light, we meet with great variety and complexity. It is well, therefore, to clear the way somewhat by reference to a not infrequent *misconception* of the nature of the action of light, in which it is spoken of as being *catalytic*. The initial phase of all photo-chemical reactions is accompanied by the actual consumption of light energy to set in motion a reaction, although it may afterwards proceed with the evolution of energy. We have seen in Chapter X. that a catalyst adds no energy to a reacting system, but merely accelerates the *rate* at which such a reaction arrives at equilibrium. Further, in many reactions, such as the decomposition of carbon dioxide by the green leaf, the reaction is actually caused to proceed in the direction *opposite* to that in which it goes naturally at the temperature of the reaction. But, in many cases, a catalyst is formed by the action of light, and this catalyst then proceeds, independently of the light reaction proper, to perform its usual function of accelerating the natural course of the reaction. In this case, contrary to that of the chlorophyll system, the net result of the change is a *diminution* in the free energy of the system.

It will, perhaps, assist the understanding of the question if we use an illustration due to Ostwald (1902, II. 1, p. 1087). The necessity of the supply of energy by light is obvious enough in that class of reactions in which the result is an increase in the energy content, but is not so clear when the final result is a decrease. Quantitative measurements show, however, that, even in the latter case, there is more liberation of energy than if the reaction had merely proceeded without light, the extra energy being that obtained from light in the initial process. Ostwald compares the system to that of a wedge-shaped block standing with its narrow edge upwards. In this position, that of "*metastable equilibrium*," the system would remain indefinitely if undisturbed, although by falling on its side energy would be given out. To cause this to take place, there is necessity for a certain expenditure of energy upon the block in order to tip it over; in this process, its centre of gravity is raised and the energy required to do this is given out again when the block falls over. Another illustration that might be given is that of a billiard ball lying in the concavity of a clock glass on the top of a tripod. Although energy would be given out by the fall of the ball, supposing that the glass were to melt away, no change takes place naturally unless the ball is first raised over the edge of the glass by the application of a small amount of energy. This energy is given out again, together with that due to its original height, when the ball falls to the table. We may speak of reactions of such a kind as being prevented from taking place spontaneously by the existence of chemical "resistance," which is removed by the energy imparted by light.

Our further considerations will be facilitated by taking the *classification* of Weigert (1911, p. 75), together with an illustration of each class. This classification is based on the net result of the reaction, which results from the action of light, not merely on the actual part played by the light energy used.

We have seen already that these reactions can be divided into two main groups, those resulting in increase of free energy and those resulting in a decrease. Each of these can be further divided. The first group may be either simple or complex, in both cases being completely reversible and similar to an electrolytic decomposition in which the electrodes become polarised, so that the reaction ceases at a certain point.

1. *Simple Reactions with Increase of Energy.*—In these cases, the reversion on removal of light takes place by the same route as the photo-chemical change.

The simplest one is the polymerisation of anthracene to di-anthracene by ultra-violet light, as investigated by Luther and Weigert (1905). The stable condition in the dark is, at ordinary temperatures, that of pure anthracene. If we start with pure di-anthracene in the dark it changes spontaneously to anthracene, at a definite rate. Light causes the formation of di-anthracene. But, since the reverse change is unaffected by light, this change of di-anthracene to anthracene proceeds at its natural rate, and this rate increases by mass action as more di-anthracene is formed by light. Under a given intensity of illumination, therefore, as much anthracene is formed by the "dark" reaction as di-anthracene is formed by light in the same time, so that a "*stationary condition*," simulating a chemical equilibrium, is arrived at. Contrary to the latter, which would be a permanent one if left to itself, this stationary condition is only maintained by the continuous inflow of light energy, which becomes transformed to heat. It should be noted that, before the stationary condition is reached, part of the light energy becomes chemical energy. Another example is that of the formation of ozone from oxygen by ultra-violet light.

A striking fact, which may appropriately be mentioned here, is that the *temperature coefficient* of a light reaction is usually much lower than that of a chemical reaction proper. This follows from the fact that the rate of the photo-chemical change depends on that of the absorption of light, which varies only very slightly with temperature. The position of the stationary equilibrium in the case of anthracene, in relation to temperature, is controlled almost entirely by the change of chemical equilibrium by temperature. The temperature coefficient of the dark reaction (chemical) is 2·8, that of the light reaction, 1·1 or less.

2. *Complex Reactions with Increase of Energy.*—These result from the combination of various purely chemical reactions with photo-chemical effects. Their reversal is by a *different route* from that taken in their production. The most interesting and important of these is that of chlorophyll and carbon dioxide, which will be treated of in a special section later. They are the most difficult class to analyse, since the various component reactions proceed both simultaneously and successively.

Those reactions resulting in diminution of free energy are always complex, as we have seen, and may be divided into two main classes: coupled and catalytic. They are non-reversible, in the sense that they do not change back spontaneously in the dark.

3. *Coupled Reactions with Loss of Energy.*—In these the products of photo-chemical change are immediately used up in another reaction. As a general scheme, we may take that of Weigert (1911, p. 36):—

$$(1)\ A \underset{\text{Dark}}{\overset{\text{Light}}{\rightleftarrows}} B. \qquad (2)\ B \xrightarrow[\text{Dark}]{} C$$

B is produced from A in the light, and would quickly return to A if it were not at once used up in the second reaction to form C. It is probable that the oxidation and reduction of alcohols by aromatic substances, observed by Ciamician and Silber, belong to this group. The important properties of the *chemical sensitisers* must be included also. A plate coated with silver bromide alone and exposed to the light belongs to our first class, so that when a certain amount of free bromine has been produced by light, a stationary, balanced condition is reached, owing to the recombination of silver and bromine, as in the dark. Such a plate would be of little use in photography, being comparatively insensitive. If, however, a substance such as gelatine is present, which combines with the bromine as it is produced, a much greater decomposition of the silver bromide takes place. There is no storage of energy, since the final system consists of brominated gelatine and metallic silver (or sub-bromide). The bromination of gelatine is associated with the giving off of energy, and the product has no affinity for silver.

An electro-chemical analogy to this group is that of an electrolytic process in which the products are used up in a reaction going on in the solution, so that depolarisation occurs and the current continues to flow.

4. *Catalytic Reactions with Loss of Energy.*—The second group of photo-chemical reactions in which there is diminution of free energy is that in which

catalysts are produced by the action of light. In these cases the action of light leads to the same products which appear in the dark under the same conditions of temperature and solvent. Light merely accelerates the process by causing the formation of a catalyst for the reaction, which then obeys the usual laws of catalysis.

It is found that the catalyst may be formed from the reagents, or one of them, by the action of light, and may then disappear on the removal of the light. Or, in other cases, the catalyst may continue to exist and exert its action for some time after the light has been taken off.

4A. *Reactions with Loss of Energy, in which a Catalyst is Formed by Light, the Catalyst lasting only as long as the Illumination, and vanishing in the Reaction.*—One of the best known of all catalytic light reactions, namely, the combination of hydrogen and chlorine under the action of ultra-violet light, belongs to this group. A great number of investigations have been made on this reaction since the first exact research by Bunsen and Roscoe (1855-1859). Details of these will be found in Weigert's monograph (1911, pp. 44-56). Under the usual conditions of experiment, the effect is found to have a latent period, the so-called "*Induction Period*," during which no combination takes place. Subsequent investigation showed that this was due to the presence of impurities, especially on the walls of the vessel used. These impurities use up the catalyst for a time. What is the catalyst? From what was said above, it is clear that light energy is used up to produce it, and it appears to be chlorine in an "activated" form of some kind.

The fact that the drier the gases are, the more slowly does the reaction proceed, suggested to Mellor (1902) that there is formation of an intermediate compound (xCl_2, yH_2O, zH_2), as in certain other cases of catalysis, such as that of molybdic acid on hydrogen peroxide and hydriodic acid, as described above (page 324). The investigations of Burgess and Chapman (1906) directed attention to the cloud formation, due to the production of condensation nuclei in the illuminated gases. Whether these nuclei are identical with the hypothetical compound of Mellor seems doubtful, and it is more probable that they do not differ essentially from other cloud nuclei formed by radiations.

Chlorine is made "active" by light for other reactions also, such as for combination with carbon monoxide, sulphur dioxide, hydrocarbons, etc. Whatever the nature of the catalyst may be, it must consist of chlorine plus light energy, and therefore act chemically as chlorine itself. Accordingly, it disappears in the reaction. In such reactions it appears that the primary action of light is to form nuclei, which start a reaction in a way analogous to that in which they cause condensation of water vapour to drops of liquid. Weigert calls them "reaction nuclei," and points out that their mode of action is like that of other heterogeneous catalysts. The reacting substances are condensed on their surfaces by adsorption, and the reaction proceeds there more rapidly as a consequence of mass action.

Compared with the reactions in which light energy is stored up, and often in considerable amount, these catalytic reactions require little energy to form the catalyst, and are, as a rule, very sensitive.

The phenomena of *optical sensitisation* belong to the present category of reactions. Light cannot act unless absorbed, and the question naturally arises whether the addition of some substance, such as a dye, to a system which is not affected by light of a particular wave length, is able to make it sensitive if the dye absorbs rays of this wave length. In point of fact, such is the case, although at first sight the reason is not obvious. The light is absorbed by the sensitiser, and must therefore produce changes in this, not necessarily in other, parts of the system. The key is given by the formation of catalysts from the sensitiser, which appear to be heterogeneous in nature and, at all events in many cases, require the presence of oxygen, "activating" it so that potassium iodide is oxidised as by ozone.

A simple experiment given by Wager (1914) shows this fact. Strips of paper containing starch are soaked in a solution of methyl violet, methyl green, eosin, fuchsin, or fluorescein, exposed to light, and then moistened with potassium iodide solution. Iodine is liberated, and stains the starch blue. It is interesting that cyanin, although bleached by light, does not give rise to active oxygen.

The mode of action of optical sensitisers seems to be of a somewhat general

nature, since certain reactions can be accelerated by practically any wave length. so long as a dyestuff is present which can absorb these particular rays.

The most important practical application of optical sensitisers is in the production of *photographic plates* sensitive to the whole extent of the spectrum. It is to be remembered that the adsorption of the dye by silver bromide does not make this itself more sensitive. It may be, as Weigert suggests (1911, p. 70), that the light absorbed by the dye makes it a better chemical sensitiser than it is in the dark, so that it takes up bromine with great avidity.

4B. *Catalytic Photo-chemical Reactions in which the Catalyst remains after the Action of Light.*—If the catalyst formed is not immediately used up in the reaction, it is clear that its activity may continue. Such a case is that of iodoform in chloroform; the iodine set free by light remains active after the light has ceased to act, and continues so for several days. Moreover, if a solution which has been exposed to the light be added to an unexposed one, decomposition of the latter sets in.

The capacity of being developed at any time after exposure, possessed by photographic plates, is another case. We cannot here discuss the nature of the latent image. The reduction-potential of the developer is not sufficiently high to affect unexposed silver bromide at any considerable rate; but, where the light has formed a catalyst, metallic silver is produced in development. It appears that the acceleration is due to adsorption of developer on the surface of the heterogeneous catalyst, by which the concentration of the former is raised and, with it, the reduction-potential (see the remarks of Weigert, 1911, p. 74).

It is clear that, in these cases of catalytic action, if we could add the catalyst in any other way than by the action of light, the result would be the same. This is not so in the three first cases of our list, where the same products of reaction as those produced by light cannot be obtained in the dark, at the same temperature, by other means.

Electro-chemical analogies for the catalytic action of light may be found in the saponification of an ester in a solution of neutral salt. The catalyst, in this case, is the alkali formed at the cathode, and it disappears by combination with the acid formed from the ester. If we take cane-sugar instead of an ester, the catalyst is the hydrogen ion formed at the cathode and it remains active after cessation of the current, provided that means are taken to prevent diffusion.

RELATION OF VELOCITY OF REACTION TO INTENSITY OF LIGHT

Bunsen and Roscoe (1862) showed that in order to produce a definite degree of darkening on silver chloride paper, the time required was inversely proportional to the intensity of the light. That is:—

$$i\,t = \text{constant}$$

where i is the intensity of the light, and t the time of action. This is known as the *Bunsen-Roscoe Law.*

When the exposure to light is followed by development, the law does not hold. Schwarzschild (1899) showed that, for silver bromide gelatine plates, the law must be expressed thus:—

$$i\,t^p = \text{constant.}$$

The value of the exponent p varies between 0·8 and 1, according to the brand of plate used. It seems probable that the exponential form of the equation may depend on the intervention of adsorption in this case, where development is made use of.

Inertia.—There is a certain minimal duration of exposure of a plate to light below which no effect is produced. This is known as the "inertia" of the plate, and appears to be related to the photo-chemical induction already referred to.

FLUORESCENCE

There remain to be mentioned some phenomena connected with the absorption of light which are not obviously photo-chemical in nature, that is, chemical changes are not immediately obvious.

It is very common to find that substances which absorb light of a particular wave length radiate it again, either at an increased wave length, or of the same wave length as that absorbed. The cases in which ultra-violet light is absorbed and given out again as visible light are the most striking. Such are: solutions of quinine salts, solid anthracene, and so on. In the cases mentioned, part of the light, as we have seen, is used for chemical change. A solution of eosin, which has an absorption band in the blue-green, gives a green fluorescence.

When we are dealing with colloidal solutions it is sometimes more difficult to state whether the phenomena observed are properly to be called fluorescence. For example, a colloidal solution of the acid of Congo-red gives an orange-red "fluorescence." The light transmitted is blue, and it seems that the particles really reflect orange-red light in the same way as the dry solid itself does, like other solids of the same colour. The light transmitted would naturally be the complementary to this colour.

In examining colloidal solutions by the Faraday-Tyndall beam, confusion may sometimes be caused by fluorescence. When this is present, the path of the beam will be illuminated, whether colloidal substances are there or not. The distinction can usually be made by interposing screens of various colours between the light and the solution, in order to absorb that part of the light, usually the ultra-violet, causing the fluorescence. The colloidal phenomenon is, of course, found with any wave length of light, provided it is powerful enough. An interesting solution to examine is fresh urine, filtered to remove any coarse particles. Examined in white light, the beam is not extinguished in any position of the Nicol prism used to observe it. But that this is due to the fluorescence of the pigment is shown by the interposition of a yellow screen to absorb the violet end of the spectrum. The Faraday-Tyndall beam is still present, and can be abolished by rotation of the Nicol prism, showing the presence of colloids.

It seems probable that both fluorescence and *phosphorescence* are really cases of photo-chemical reactions with storage of light energy. Phosphorescence is the giving off of light, not only during illumination, but for a longer or shorter time afterwards. Weigert (1911, p. 26) suggests that a substance A is changed to a substance B, with storage of light energy. The spontaneous return of B to A is associated with the giving out of this energy, again in the form of light. The view is supported by the fact that fluorescence can be changed into phosphorescence at the temperature of liquid air, owing to the reverse reaction being retarded. Phosphorescence itself may be abolished at this temperature, but appears on warming.

The use of fluorescence in the observation of living tissues under the microscope, has been described above (page 9).

CHEMI-LUMINESCENCE

When bodies are heated gradually they may be seen to begin to give off light when a certain temperature is reached. This light consists, when it first appears, at a temperature of somewhere about 1,000°, only of the longer wave lengths. As the temperature rises, shorter and shorter wave lengths are progressively added. The temperature called "white-heat," as is well known, is very high. Thus light of a particular wave length is associated with a particular temperature in the case of this form of radiation. But light is given of by many chemical reactions at a temperature much below that corresponding to the wave length of the light emitted, supposing it to have been produced by rise of temperature only. This phenomenon is known as chemi-luminescence and is not uncommon. It may be seen by taking a mixture of 10 c.c. of 10 per cent. pyrogallol, 20 c.c. of potassium carbonate, and 10 c.c. of commercial formalin. Add, in the dark, 30 c.c. of 30 per cent. hydrogen peroxide. An orange-red glow, accompanied by considerable foaming, will be seen. A list of the reactions in which similar emission of light can be seen, will be found in the paper by Trautz (1905). The reactions in which it occurs are themselves sensitive to light, and the wave length of this light is the same as that which is emitted in the reaction. Thus the system is set into vibration of its own particular rate by the chemical reaction itself (see Nernst's book, 1913, p. 815).

We may also speak of such reactions as being cases of direct conversion of

chemical energy into light energy, without passing through heat. In the same way, certain photo-chemical reactions are direct conversion of light energy into chemical energy.

The bearing of these phenomena on the emission of light by organisms will be seen later.

The light emitted by the Welsbach mantle appears to be of a shorter wave length than that corresponding to the temperature of the Bunsen flame. It is difficult, however, to determine accurately what is the temperature of the flame. That of a body in it depends on the ratio of its powers of emission and absorption of radiation. For example, a bead of sodium phosphate on a loop of platinum wire is barely luminous in the Bunsen flame, while the platinum glows brightly.

PHOTO-ELECTRIC EFFECTS

When a ray of light falls upon a metallic electrode, the potential of this latter is changed.

The discharge of a charged electroscope by ultra-violet light, the Hallwachs effect, will be referred to later.

The change in the resistance of selenium on exposure to light has been made practical use of in the transmission of pictures by electric current.

Two kinds of explanation have been given of the origin of the potential difference in photo-electrical cells. One may say that the light which falls upon an electrode of silver chloride raises the tension of chlorine, and secondarily the potential of the electrode, or that electrons are torn off from their combination by the increased kinetic energy.

For further information the reader may consult the book by Allen (1913).

THE CHLOROPHYLL SYSTEM

We are now in a position to discuss with more profit the action of chlorophyll in the decomposition of carbon dioxide and evolution of oxygen, perhaps the most interesting of all natural phenomena.

History.—There are three important dates to be noted.

Priestley (1774, pp. 89-92) observed that air "spoilt" by mice, that is, incapable of supporting animal life, was made good again by allowing green plants to remain in it for some time.

Ingenhousz (1780) showed that this action of green plants only takes place in the light. He says: "The light of the sun is alone capable of producing in the leaves that movement which can develop dephlogisticated air" (that is, oxygen): "as soon as the light ceases to act on the leaves, their operation ceases at the same time, and another of a different nature commences." (Translated from p. 17 of the French edition.) He also shows clearly that it is not the heat that is responsible for the result (p. 38). In Fig. 177 I have produced his little allegorical initial picture of how light purifies the air.

Senebier (1783, see pp. 410-442 of his book, 1788) showed that the chemical change involved is the conversion of fixed air into dephlogisticated air, that is, carbon dioxide into oxygen. De Saussure (1804, Chap. 2) investigated the phenomena quantitatively.

General Nature of the Reaction.—As already mentioned, the process, taken as a whole, results in a large storage of light energy, and is one of those complex reactions which are the most difficult to investigate. We have to deal with several reactions chemically coupled, some sensitive to light, others apparently not: together with both optical and chemical sensitisation.

As is well known, it is the presence of the pigment, chlorophyll, which enables the reactions to take place. Those parts of variegated leaves which are devoid of chloroplasts, although otherwise similar to the green parts, are incapable of photosynthesis, as it may be called for convenience.

The Chemistry of Chlorophyll.—Although much very interesting work has been done on the chemical constitution of chlorophyll, especially by Willstätter, it must

be confessed that, valuable as it is, it has not, *as yet*, thrown much light on the problem before us. The brief account which follows is taken from the book by Willstätter and Stoll (1913).

The method of preparation will be found on p. 133 of the book. The most important point is the first extraction of dried leaves (say of nettle or elder) with 80 per cent. acetone, which does not extract wax and fatty substances.

Stokes (1864) had already pointed out that what is usually known as chlorophyll consists of a mixture of two green substances, and that it is accompanied in leaves by two yellow pigments. Willstätter confirms this statement, and calls the two chlorophylls *a* and *b*: these are identical in all plants, and contain magnesium and nitrogen, but neither iron nor phosphorus. The yellow pigments, carotin and xanthophyll, are free from nitrogen.

FIG. 177. ALLEGORICAL PICTURE REPRESENTING THE EFFECTS OF THE LIGHT OF THE SUN ON GREEN PLANTS AND THE PURIFICATION OF THE AIR THUS EFFECTED BY THEM.

(Ingenhousz, 1780. Initial figure.)

As to the quantity of these four substances to be obtained from leaves, we find the following data: From 1 kg. of dried elder leaves (= 4 kg. of the fresh leaves) were obtained:—

8·48 g. of chlorophyll, consisting of
6·22 of *a*-chlorophyll, and
2·26 of *b*-chlorophyll.

Together with

1·48 g. of carotinoids (yellow pigments), namely,
0·55 g. of carotin
0·93 g. of xanthophyll.

The two chlorophylls are separated by partition between methyl alcohol and petroleum ether. The latter takes up *a*-chlorophyll; the former, *b*-chlorophyll, since it is insoluble in petroleum ether. Even *a*-chlorophyll is difficult to dissolve in pure petroleum ether. It has a blue-green colour, with red fluorescence. By rapid dilution with water a colloidal solution is obtained, devoid of fluorescence. Chlorophyll-*b* has a green or yellow-green colour in solution. There is a slight difference between their absorption spectra, as will be seen later. The action of acid on the *a*-substance gives an olive-green derivative, on the *b*-substance, a red-brown one. Chlorophyll-*b* is an oxidation product of the *a*-substance, containing an extra oxygen atom in place of two hydrogen atoms. Both are micro-crystalline. They are adsorbed by charcoal, and cannot be extracted again by petroleum ether, but by pyridine, no doubt a question of relative lowering of surface tension. Their behaviour to reagents is essentially similar, and the word chlorophyll will be used below to include both.

Further information of their constitution is obtained by treatment with reagents.

The Action of Acid on chlorophyll directly is to separate magnesium from it, forming a derivative called "phæophytin." The series of derivatives devoid of magnesium are called in general "phytins." The magnesium in chlorophyll is in organic combination, and by treatment of the magnesium-free phæophytin with Grignard's reagent (magnesium-methyl-iodide), the magnesium is replaced and chlorophyll obtained again.

On *saponification* by alkali, phæophytin shows itself to be an ester, the acid contains nitrogen and has thirty-four carbon atoms, while the alcohol (called "phytol") is free from nitrogen and is a monatomic alcohol of the composition $C_{20}H_{40}O$.

Phytol appears to contain a number of groups $-\underset{\underset{\displaystyle CH_3}{|}}{CH}-$ in a chain. It is colourless, and of less interest than the coloured acid component.

Chlorophyll is, therefore, a *phytol-ester* of a nitrogenous acid chlorophyllin, which contains magnesium in organic combination. The acid of the phæophytin from chlorophyll *a* is olive-green in neutral solvents, and called phytochlorin. That from chlorophyll *b* is red in neutral solution, and called phytorhodin. Since it is an ester, it is not surprising to find that chlorophyll is accompanied in the leaf by an enzyme (a lipase or esterase), which is active in alcoholic solution. In extracting leaves with alcohol, "alcoholysis" of the chlorophyll takes place, and ethyl takes the place of phytyl, forming an ethyl-chlorophyllide. The enzyme acts synthetically, as would be expected, and forms chlorophyll in a concentrated solution of phytol and the chlorophyll acid. The mono-carboxylic acid, which is split off by alkali from chlorophyll, is called "chlorophyllin," and contains magnesium. Its derivatives are the "phyllins." These latter are produced by further action of alkali, which splits off carboxyl groups. These phyllins still contain the magnesium, which is combined with the nitrogens of four pyrrol groups. To split off the magnesium from them acid is necessary, and we then obtain the "porphyrins." Thus:—

Chlorophyll *a*

(by acid) ↙ ↘ (by alkali)

Magnesium + phæophytin *a* (by alkali) ↓ phytochlorin + phytol

phytol + chlorophyllin (contains Mg) (by alkali at 200°) ↓ phyllins (by acid) ↓ porphyrins + Mg

The porphyrins are of interest, because they serve to bring into connection *chlorophyll and hæmoglobin*. The blood pigment seems to be a derivative of a substance which contains iron united to four pyrrol groups in a way similar to the magnesium of chlorophyll (see Küster's paper, 1908, and the book of Willstätter and Stoll, 1913, pp. 42 and 39). Hoppe-Seyler (1880, p. 201) described a compound, which he called "phylloporphyrin," obtained from chlorophyll, which gave the same absorption spectrum as the hæmatoporphyrin derived from hæmoglobin. Similar pyrrol derivatives have been obtained from both.

If one of the porphyrins, obtained by the action of acid on phyllins, be heated with soda-lime, "ætioporphyrin" is formed; it turns out to be the same substance as that which is formed from hæmatoporphyrin by similar treatment. Now hæmatoporphyrin is formed from hæmatin, which is hæmoglobin minus its protein component, by the removal of iron by acid, as magnesium is removed from phyllin by acid. To form hæmoglobin, hæmatin combines with a protein, globin; to form chlorophyll, phyllin, or a carboxylic acid derived from it, combines with an alcohol, phytol, to form an ester. Chlorophyll, however, loses its magnesium more readily than hæmoglobin does its iron.

As regards the pyrrol constituents of the two pigments, information is to be obtained by oxidation. Küster (1909) obtained, by oxidation of hæmin (= hæmatin hydrochloride), an imide of an acid, which he called *hæmatinic acid*. Willstätter finds that chlorophyll behaves in a similar way. The porphyrins

from it gave an imide of hæmatinic acid, together with methyl-ethyl-maleic imide. Further, Nencki and Zaleski (1901) found that hæmin, on reduction, gave *hæmo-pyrrol*, which is, according to Küster (1908) and Piloty (1909), a dimethyl-ethyl-pyrrol. Willstätter obtains this substance from chlorophyll also, and finds it to be a mixture in both cases of three isomeric dimethyl-ethyl-pyrrols.

From information given me by Prof. Willstätter, I understand that he does not regard the similarity in constitution between chlorophyll and hæmoglobin as being of any great significance. The mother substances were probably at hand, and compounds with the properties of the two pigments respectively being required, if one may use the expression, these pyrrol derivatives were made use of.

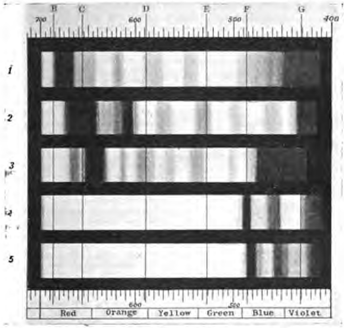


FIG. 178. ABSORPTION SPECTRA OF CHLOROPHYLL AND ASSOCIATED PIGMENTS.—Scale of wave lengths at top and bottom. Fraunhofer lines marked at the top. The colours of the regions of the spectrum indicated at the bottom.

1, Nettle leaf, living. Chlorophyll in colloidal state (Willstätter und Stoll, p. 62).
2, *a*-chlorophyll in ether (do., p. 170).
3, *b*-chlorophyll in ether (do., p. 171).
4, Carotin in alcohol (do., p. 246).
5, Xanthophyll in alcohol (do., p. 246).

In the leaf, according to Willstätter, chlorophyll probably exists as an adsorption compound with a colloid, but not combined with a lipoid, as some have stated.

The two *yellow pigments* are both unsaturated and autoxidisable, that is, they spontaneously oxidise in the air. They are nitrogen-free. Both pigments have two absorption bands in the blue and blue-violet (see Fig. 178).

The one, *carotin*, is an unsaturated hydrocarbon ($C_{40}H_{56}$). It is soluble in petroleum ether and is identical with the "lutein" of the corpora lutea of mammals.

It is isomeric with the "lycoperdin" of the tomato. As we shall see later, it may play a part in the decomposition of carbon dioxide by the chloroplast system.

Xanthophyll is an oxide of carotin ($C_{40}H_{56}O_2$). It is insoluble in petroleum ether, but soluble in methyl alcohol. It is isomeric with the "lutein" of the fowl's egg, which has no relation to cholesterol, as had been supposed.

ABSORPTION OF LIGHT BY CHLOROPHYLL

In Fig. 178 the absorption spectrum of chlorophyll, in its two forms, is given. The most striking and characteristic appearance is the dark band in the red, which is divided into two in chlorophyll-*b*. As will be noted, the chief absorption is in the longer wave lengths and is practically in the position of the maximum energy of the solar spectrum, during the greater part of the day. S. P. Langley's measurements of the position of maximum energy gave 650-666 $\mu\mu$ for high sun. The latter number is easy to remember, as Timiriazeff points out, being the "number of the beast." The middle of the chief band of chlorophyll-*a* is, in solution in ether, at 662 $\mu\mu$. In colloidal solution in 1 per cent. acetone, the band is shifted towards the red, so that its maximum is at 678 $\mu\mu$. This is the same as that of its natural state in the leaf. The maximum energy of solar radiation, also, would be for the greater part of the day nearer the red than the figure of Langley. Chlorophyll has a considerable absorption in the blue also, but practically none in the infra-red, nor in the yellow-green, not much in the ultra-violet.

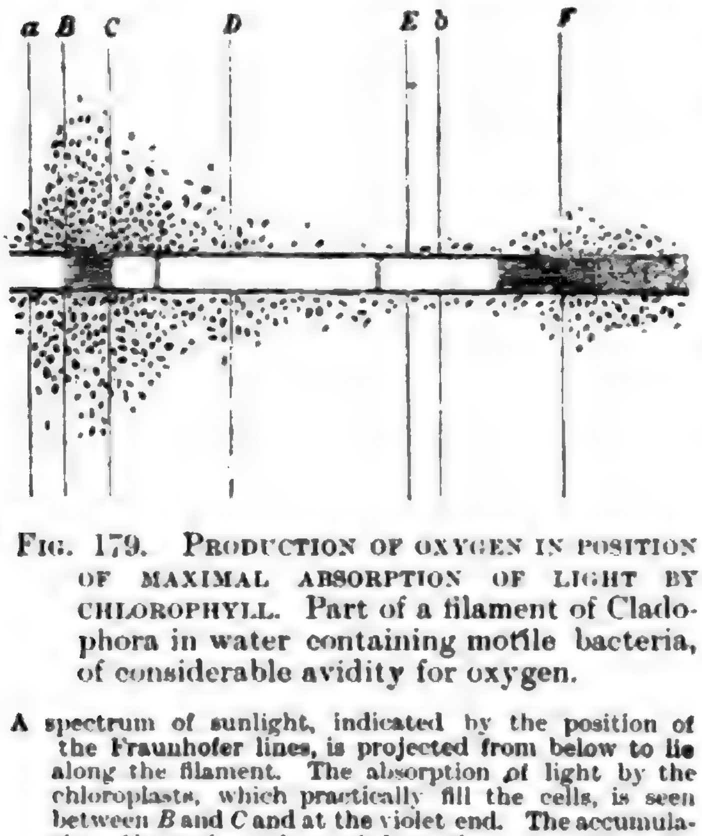


FIG. 179. PRODUCTION OF OXYGEN IN POSITION OF MAXIMAL ABSORPTION OF LIGHT BY CHLOROPHYLL. Part of a filament of Cladophora in water containing motile bacteria, of considerable avidity for oxygen.

A spectrum of sunlight, indicated by the position of the Fraunhofer lines, is projected from below to lie along the filament. The absorption of light by the chloroplasts, which practically fill the cells, is seen between *B* and *C* and at the violet end. The accumulation of bacteria at places of absorption, especially in the red, shows that oxygen is being produced there. Magnified 188 times.

(Engelmann, 1882, 1, p. 195.)

It is remarkable that some of the earlier observers believed that their experiments showed that the maximum photo-chemical change occurred in the yellow-green region, in which the absorption of light energy is minimal. This would be a difficulty in view of Grotthus's law, and later observations, especially by Engelmann, showed it to be due to incorrect estimation of the absorption of the screens used.

A striking demonstration of the fact that the maximum evolution of oxygen is at the place of the greatest absorption of light was given by Engelmann (1882, 1). This was done by the use of a bacterium, which was very sensitive to oxygen. Water containing these organisms was placed, along with a thread of a green alga, on the stage of a microscope. In the same plane, and along the thread of alga, a minute spectrum was projected by means of a spectroscopic arrangement beneath the stage. It was seen that the bacteria accumulated precisely at the places where the absorption bands of chlorophyll were situated (see Fig. 179). Another experiment, showing the same fact, is due to Timiriazeff (1903). A leaf on a plant is deprived of its stored starch by being kept in the dark. A small spectrum is projected on to its surface and, after some time, the leaf is decolorised by alcohol and treated with iodine. The absorption bands of chlorophyll are then found to be mapped out by the action of the iodine on the starch, which has only been formed in these places (Fig 180). Measurements have also been made, spectrophotometrically, of the absorption of light in different regions of the spectrum and compared with the photo-chemical effect. There are two maxima shown, but, when the curve is corrected for the normal spectrum, in which equal abscissæ correspond to equal differences of wave length, the second maximum in the blue end is found to be comparatively unimportant. From

Engelmann's results, it appears that, in proportion to the light absorbed, the efficiency of the various regions of absorption is the same. In other words, so long as light is absorbed, it does not seem to matter what the wave length is. Kniep and Minder (1909), also, have compared the carbon assimilation with the relative amount of energy of the light absorbed in different parts of the spectrum and state that it is in direct proportion. This fact seems to suggest that the actual pigment itself is merely an optical sensitiser, since there is no relation between its particular absorption bands and the photo-chemical change.

Lasareff (1907) similarly showed that the bleaching of certain dyes is in proportion to the light energy absorbed, whatever the colour of the light. It is obvious, however, that, in so complex a system as the living cell, an exact agreement is not to be expected; the oxygen, for example, may be partly used up by the protoplasm, and structures other than the chloroplasts absorb light.

We may take it, then, that the maximal effect of the chlorophyll system is in relation to that part of the spectrum which is absorbed most.

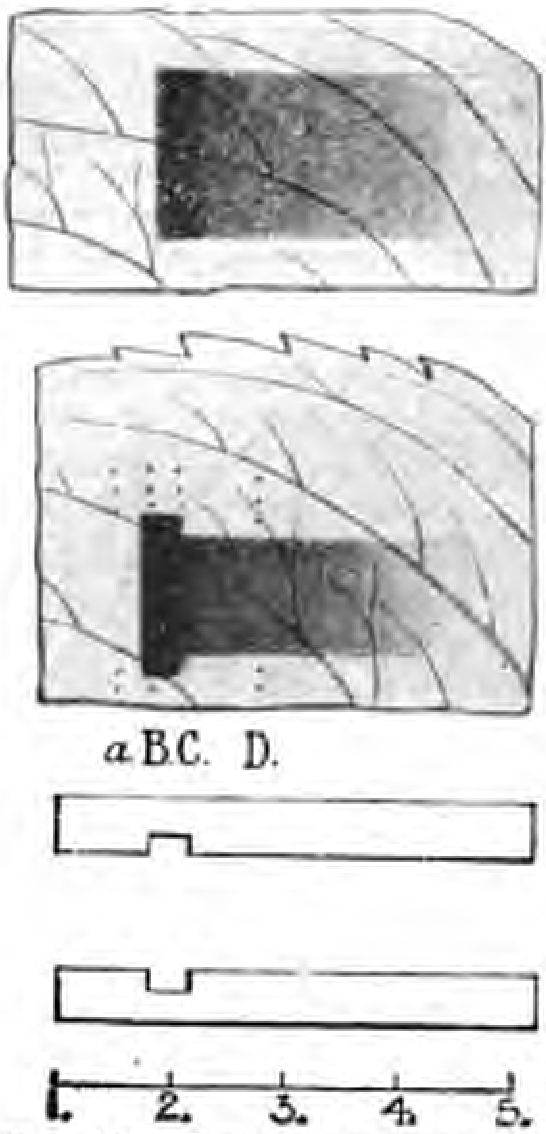


FIG. 180. PRODUCTION OF STARCH IN THE SPECTRUM.

Hydrangea leaves, still attached to the plant, have been deprived of starch by keeping in the dark. They have then projected upon them a small solar spectrum for five to six hours. Subsequent treatment with iodine, in the usual way, shows a picture of the absorption spectrum of chlorophyll in the blue "compound" of iodine and starch. The lower piece of leaf has been partially covered with a screen, represented below it, in such a way that the wider part of the aperture corresponded with the region of the spectrum between the lines *B* and *C*.

(Timiriazeff, 1903, p. 434.)

THE STRUCTURE OF THE CHLOROPLAST

In view of the results obtained by various observers with solutions of chlorophyll extracted from the leaf, it is important to remember that, *in situ*, this pigment is closely associated with other substances in the granules known as chloroplasts. It appears to form a thin, highly concentrated layer on the surface of these bodies and is practically solid (see the paper by Timiriazeff, 1903, p. 455), or in the colloidal state, since it shows no fluorescence. As we saw (page 562), its spectrum in the leaf is the same as that of the colloidal solution. Owing to its close association with the complex system of the chloroplast, it is scarcely to be expected that it would be possible to obtain the complete photo-chemical change in preparations containing chlorophyll alone. Miss Irving (1910) found that, if a seedling be grown in the dark and then placed in light, chlorophyll may be produced in the cells before they have developed the power of photo-synthesis. At the same time, it is obviously of interest and importance to commence with the action of pure chlorophyll and, if possible, add the other constituents of the system later.

In this connection, we may remember the importance of the *structure* of the cell, not only for oxidation processes, about which we shall have to speak later, but also for the re-establishment of the contractile system of muscle, with addition of energy, a process more analogous to that of photo-synthesis.

THE PHOTO-CHEMICAL REACTIONS OF THE CHLOROPHYLL SYSTEM

The final result of the process may be represented by an equation such as:—

$$xCO_2 + xH_2O + \text{light energy} = C_xH_{2x}O_x + xO_2,$$

but this naturally gives us no indication as to how it is brought about.

The fact that light energy is stored up shows at once that we have to deal with a process that is *not a catalytic* one. The reduction of carbon dioxide at ordinary temperatures is effected against chemical forces. As Weigert points out (1911, p. 99), this indicates that chlorophyll itself takes part in the reaction and that the considerable increase in potential which occurs is due to the interaction with other parts of the chloroplast, as indicated above. This raising of potential is a common phenomenon in physiological processes (see the paper by Weigert, 1908, p. 464).

In living organisms, as we know, the process is a *reversible* one, since carbohydrate is oxidised with production of carbon dioxide and water, but it is not necessary that the same intermediate stages should be passed through; in fact it does not seem probable that they are. If, however, we take the simplest form of the equation given above, making $x=1$, formaldehyde is one of the products on the right-hand side, and this is oxidised by oxygen, at all events by "active" oxygen, giving out light as a phenomenon of chemi-luminescence (see page 557 above). According to Trautz (1905, p. 101), this light is of a reddish colour, in fact, of the same wave length as the position of the main chlorophyll absorption band. Thus, the equation might be written, with the inclusion of light as a part of the reversible system:—

$$CO_2 + H_2O + \text{light energy of definite wave length} \rightleftarrows HCHO + O_2.$$

It was suggested by von Baeyer (1870) that formaldehyde is the first product of photo-synthesis, and Usher and Priestley (1906) found that an aldehyde is to be detected as a product of the action of light on films of chlorophyll in the presence of moist carbon dioxide. There is also reason to expect formaldehyde to be produced, since Bach (1893) obtained formic acid by the action of light on carbon dioxide in presence of solutions of uranium salts, and Moore and Webster (1913) have obtained formaldehyde by the action of ultra-violet light upon colloidal solutions of uranium hydroxide or ferric hydroxide. Moreover, formaldehyde is readily polymerised to higher carbohydrates. Loew (1889) obtained, by the action of magnesium oxide and lead on formaldehyde at 60°, a sugar which he called *formose*; this was afterwards shown by Emil Fischer (1890) to be inactive fructose. Berthelot and Gaudechon (1910) obtained formaldehyde by exposing a mixture of carbon dioxide and hydrogen, or water and carbon monoxide, to ultra-violet light, and Walther Loeb (1905) by exposing moist carbon dioxide to the silent electric discharge. In both cases, a series of intermediate reactions took place, and the conditions are, perhaps, rather far from those of the green leaf, although, as we have seen, the photo-chemical process, in Luther's view, is fundamentally an electric one. The alternating electric field of the silent discharge is not very far removed from that of light, but, of course, the frequency of the alternations is very much less.

Now, if the aldehyde in Usher and Priestley's experiments were actually derived from the carbon dioxide present, a great step would have been taken, but we have already seen reason to doubt whether such a reaction is possible by the aid of chlorophyll alone. Schryver (1910) showed that an aldehyde is only to be obtained from chlorophyll after it has been exposed to light, but that the production does not depend on the presence of carbon dioxide. Recent work by Wager (1914) and by Warner (1914) confirm this result as to the production of some aldehyde from chlorophyll by light in the absence of carbon dioxide; they find also that oxygen is necessary, and that it is used up. Wager is doubtful whether the aldehyde formed is formaldehyde, since the colour given with Schryver's reagent is different from that given by pure formaldehyde. The aldehyde produced under these conditions is a result of the photo-chemical oxidation of chorophyll itself, which becomes bleached. Wager shows that the amount of aldehyde produced is proportional to the amount of absorption in the different regions of the spectrum. He could not detect any disappearance of carbon dioxide when chlorophyll films were exposed to light in its presence, but admits the possibility that his method might not have been delicate enough to detect a minimal disappearance. An important fact shown is that, when

chlorophyll is oxidised by such reagents as hydrogen peroxide or potassium permanganate, an aldehyde is formed. Similar results were arrived at by Warner independently. Both investigators also found that an oxidising substance is produced at the same time as the aldehyde. This oxidising agent is of a peroxide nature, and Warner makes the statement that the bleaching of chlorophyll is due to hydrogen peroxide, as had been stated by Usher and Priestley previously. Wager, on the contrary, was unable to obtain any of the usual reactions of hydrogen peroxide; but the experimental results of Usher and Priestley are difficult to interpret otherwise. They coated a plate with gelatine containing the enzyme, catalase, obtained from the liver. This enzyme decomposes hydrogen peroxide with evolution of gaseous oxygen, and so far as is known, does not so decompose other peroxides. There is, however, another enzyme, peroxidase, which decomposes other organic peroxides, as well as hydrogen peroxide, but does not cause the production of gaseous oxygen, so that it could not account for the following result. The film of gelatine, containing catalase, was coated again with a film of chlorophyll, and exposed to light in the presence of carbon dioxide. The gelatine film, after a time, was found to be full of bubbles of gas, while the chlorophyll remained unbleached. The fact can only be explained by the rapid diffusion of hydrogen peroxide into the gelatine film, and its decomposition there before it had time to affect the chlorophyll to any perceptible extent.

These results are very suggestive in view of what has been said above with regard to the phenomena of optical sensitisation, in which we find frequently a similar activation of oxygen, associated with the partial decomposition of the sensitiser. Although it appears that the product of the action of light on a dye acts, in the ordinary cases, as a catalyst, it does not seem necessary to assume this in the case before us, since the results can be explained by the oxidation of part of the pigment by the peroxide produced, with the production of an aldehyde. At the same time, there is no reason to deny the existence of a catalytic process as part of the phenomenon; in fact, that part with which we are now concerned is not one in which energy is stored.

The point to which we have arrived seems to be this. The aldehyde which is split off from the chlorophyll system must have been derived from some constituent of the chlorophyll, probably the phytol, since it is formed by light irrespective of the presence of carbon dioxide. The experiments just related, therefore, give us no information as to the most difficult part of the problem, namely, how formaldehyde is produced from carbon dioxide, or if such is the case.

As to this, Hoppe-Seyler (1881, p. 139) suggested the hypothesis that chlorophyll combines with H_2CO_3 ($=CO_2+H_2O$); this compound is supposed "to fall apart, under the influence of light, in such a way as to yield chlorophyll (or the catalyst contained therein), oxygen and a third product, either sugar or a substance from which sugar may be formed." We have seen, on the other hand, that the main process, as a whole, is not a catalytic one, and the assumption of a chemical compound of chlorophyll with carbon dioxide is, at present, devoid of proof. We may perhaps regard the association of carbon dioxide and water with chlorophyll as some kind of a physico-chemical system analogous to that of muscle. By the taking up of light energy, this system is converted into one in which the chemical potential of the carbon dioxide plus water is raised to that of formaldehyde plus oxygen, or hydrogen peroxide. The formaldehyde may possibly be combined chemically with the chlorophyll, or it may be merely associated in some way, such that it is split off by the subsequent action of light and oxygen. Of course, the processes proceed simultaneously under natural conditions. We may represent it thus, in diagram, putting (C) for chlorophyll:—

$$H_2O\,(C)\,CO_2 + \text{light energy} \longrightarrow (C)\,CHOH + O_2 \qquad (1)$$

$$(C)\,CHOH \longrightarrow (C) + CHOH \qquad (2)$$

It must be assumed that the oxygen is molecular, inactive, oxygen and given off as gas, so that the formaldehyde escapes oxidation. The first stage does not appear to have been obtained outside the living leaf and it probably requires the complex mechanism of the chloroplast. What this mechanism is requires further investigation

Willstätter and Stoll (1913, p. 25) make the following suggestion. Carbon dioxide, attracted to the chlorophyll system by virtue of the magnesium it contains, is reduced by chlorophyll-*a* (by the agency of light energy), which itself becomes chlorophyll-*b*. As we saw, chlorophyll-*b* is an oxidation product of chlorophyll-*a*, containing one atom more oxygen. Two molecules of chlorophyll-*b* might then give off a molecule of oxygen, and become chlorophyll-*a* again. It is further suggested that this removal of oxygen may be the function of carotin, which thereby becomes xanthophyll. A reducing enzyme finally converts xanthophyll into carotin again. But this naturally is, at present, purely hypothetical.

It will be seen how far we are from understanding the process.

Usher and Priestley (1906) think that hydrogen peroxide is the immediate source of the *oxygen given off* in photo-assimilation; this peroxide is decomposed by catalase, present in all green leaves, with evolution of molecular oxygen. It is possible, however, that the hydrogen peroxide detected by them is only the product of the second stage of our hypothetical process, which takes place *in vitro* and is concerned with the splitting off of formaldehyde from its temporary association with chlorophyll. It may even have nothing to do with the real carbon dioxide assimilation, being possibly concerned with the action of the chlorophyll as an optical sensitiser. It is clear, in any case, that, in the reduction of carbon dioxide, oxygen must be dealt with in some way, and the final net result is that a volume of oxygen equal to that of the carbon dioxide is given off.

If formaldehyde is taken up in any way by chlorophyll, the latter is shown to be a *chemical sensitiser*, as well as an optical one.

Fenton (1914) finds that, under appropriate conditions, formaldehyde and hydrogen peroxide combine to form a compound $2H.CHO.H_2O_2$, which is crystalline and fairly stable at ordinary temperatures. It takes fire if brought into contact with reduced iron or platinum black. It is decomposed by sunlight.

After all, it seems most likely that the aldehyde which results from the action of light plus oxygen on chlorophyll *in vitro* may come from actual *decomposition* of the molecules of chlorophyll, as it does from other organic substances. In such a case it would have no relation to the photo-synthetic process, and be a purely artificial phenomenon. Curtius and Franzen (1912) obtained from leaves $\alpha\beta$-hexylene-aldehyde, $CH^3 - CH^2 - CH^2 - CH = CHO$. The production of such higher aldehydes suggests a possible origin from the phytol of chlorophyll.

Since chlorophyll is an optical sensitiser and these act by formation of catalysts (Weigert, 1911, pp. 64-70), the possibility must not be disregarded that it may have no other function. If this be so, the formation of formaldehyde from carbon dioxide and water would only be possible in the complex system of the chloroplast, as already suggested above. Although there are certain difficulties in this view, such as the peculiar chemical nature of chlorophyll itself, as an organic magnesium compound, it seems by no means unlikely that it may turn out to be the correct one. If so, the photo-chemical reaction by which carbon dioxide and water are converted into formaldehyde and oxygen, with the taking up of light energy, is effected by other constituents of the chloroplast, perhaps with the aid of iron, as pointed out by Moore (1914), and that the use of the optical sensitiser, chlorophyll, is to enable a sufficient supply of light energy to be available.

As to the further change of formaldehyde into *sugar and starch*, this readily takes place under ordinary chemical conditions, as stated above (page 564). At the same time, if the process were as simple as this in the leaf, it would seem that formaldehyde should serve as a means of formation of starch, independent of light. Now, experiments by Miss Baker (1913) show that this is not so, formaldehyde does not serve as carbon food for plants in the dark, although it does so in the light. It is probable, therefore, that light accelerates the polymerisation, so that there is never much free formaldehyde present at one time, thus avoiding the well-known toxic effects of this substance. The energy change is small in the process of polymerisation of formaldehyde and a catalyst may be produced in the chlorophyll system under the action of light, thus adding another factor to the complex system.

Timiriazeff (1903, p. 455) has made an interesting calculation, on the basis of the measurements of Horace Brown and others, to be given presently, of the actual amount of light energy absorbed by chlorophyll. The result is that, if all the

light energy were converted into heat in the chlorophyll itself, a temperature of 6,000° C. would be obtained. Of course, the energy is converted into chemical work, without passing through heat, but the calculation gives us an idea of the intensity of the energy changes brought into play. It may be noted that carbon dioxide is dissociated into carbon monoxide and oxygen at about 1,200°, but this fact would not assist the comprehension of the photo-chemical process, even if we admit that such a temperature might be attained locally in the chloroplast, since the union of carbon monoxide and hydrogen to produce formaldehyde requires the action of light.

The *reduction of carbon dioxide* can, nevertheless, be effected by certain protoplasmic systems by the aid of *chemical energy without light*, so that the photosynthetic process is not to be regarded as an altogether singular one. Other forms of energy, besides that of light, can be utilised by certain organisms for the purpose. For example, there are some bacteria which can use the energy obtained by the oxidation of hydrogen to reduce carbon dioxide for their own carbon needs. Suppose we grow these bacteria in a closed vessel, containing hydrogen and oxygen, we find that combustion of the gases to form water takes place. If carbon dioxide is also present, it simultaneously disappears and the carbon is assimilated by the bacteria. If hydrogen and carbon dioxide alone are present, there is a slight disappearance of both, but very little (see also Winogradsky, 1904, and Beijerinck, 1904).

Electrical Changes.—Such have been described in the green leaf in consequence of illumination. The work of Haacke (1892) and of Waller (1900, 2) may be mentioned. The effects appear to be connected with the photo-synthetic process, since they are absent if the light has already been deprived of the rays absorbed by chlorophyll by passing through another green leaf previously. These effects are nearly as great when red light is used as when white light is used. Waller has shown that removal of carbon dioxide abolishes the response, and that it can be brought back by adding carbon dioxide to the atmosphere in which the leaf is situated. The fact of an electrical response is of interest in connection with the electronic theory of photo-chemical change, described above (page 552), but cannot as yet be explained. Harvey Gibson and Titherley (1908) have suggested an electrochemical theory of chlorophyll assimilation on the basis of these electrical effects.

Red and Brown Seaweeds.—The absorption of light by chlorophyll, as we have seen, is such as to make the best use of the light available. But a green pigment is, of course, transparent to the green rays, which preponderate under water, so that it would be inefficient in that situation. Accordingly, as Engelmann has pointed out (1882, 2), we find, in the seaweeds, red and brown pigments corresponding to chlorophyll and having the same function, but able to absorb effectively the green light available. For example, the red seaweeds show a maximum of carbon assimilation in the green and, spectrophotometrically measured, they show the greatest absorption in the same region, although there is also considerable absorption between the lines B and C, where the chief band of chlorophyll lies. The minimum of absorption is in the orange between C and D (p. 220 of the paper referred to). This fact serves to illustrate the function of chlorophyll as an optical sensitiser; the same effect is produced by light of various wave lengths, provided that it is absorbed.

In certain cases, to which Engelmann has given the name of "*complementary chromatic adaptation*," we find that a pigment is actually formed under the action of coloured light and that the pigment has a colour which is complementary to that of the light to which the organism is exposed, so that this light is then absorbed. The alga, *Oscillaria sancta*, as shown by the work of Gaidukov (1902), occurs in several colours between reddish purple and blue-green. If cultures are made, say, of the reddish-purple variety, we find that under red light a green pigment is produced. If we take the green variety, it becomes reddish under green light, brownish yellow under blue light, and so on. The general colour of a mixed culture thus tends to become complementary to that of the light under which it is grown. The work was done with great care and spectro-photometer curves of the various pigments were compared with those of the light under which they made their appearance.

The phenomena seem to be related to the colours assumed by Wiener's (1895) *photochlorides* under the action of light of various colours; but the case with which we are concerned shows the opposite effect, not the production of a similar colour. The production of a substance of a colour *similar* to that of the light acting is well shown by Stobbe's (1908) *fulgides*; orange light produces an orange dye, blue light, a blue one. Thus, a substance is formed which does not absorb the light acting, so that no further change takes place; although, if the product is exposed to light of a different wave length from that under which it was produced, further change is effected since the light is absorbed.

FACTORS AFFECTING PHOTO-SYNTHESIS

Temperature.—A pure photo-chemical process has a low temperature coefficient, like that of physical phenomena, as we saw above (page 42). The photo-synthesis of carbon dioxide, not being a simple photo-chemical process, has a high one, especially at low temperatures; it is 6 for the 10° between $-5°$ and $+5°$, but only 1·76 between 20° and 30°. Certain "limiting factors," to be referred to below, complicate the measurements, so that, under ordinary conditions, the rate of the reaction is the same between 3° and 30°.

The coagulation of the chloroplast by heat takes place at a lower temperature than that of protoplasm in general.

A high temperature coefficient at low temperatures, although only a more pronounced effect of a general phenomenon (see page 42), is of frequent occurrence in complex physiological processes. For example, that of the geotropic reaction is 6·5 for the interval from $-10°$ to 0°.

Anæsthetics.—The complex nature of the process is shown by the fact that chloroform, even in traces, stops it; 0·002 c.c. per litre of air suffices.

Limiting Factors.—The importance of these factors as regards the velocity of the reaction has been emphasised by Frost Blackman (1905). They may be temperature, light, or access of carbon dioxide. It will be clear that, if the amount of carbon dioxide present is less than the system can deal with under a certain intensity of illumination, no increase in rate will be obtained by increasing the light, but will be obtained if the carbon dioxide is increased. This is, in fact, the usual state of affairs. Under ordinary conditions of good illumination, the leaf can deal with considerably more carbon dioxide than is able to diffuse to the chloroplasts through the stomata and intercellular passages (see Brown and Escombe, 1905).

The effect of accumulation of sugar is to cause the stomata to close and cut off the supply of carbon dioxide.

THE EFFICIENCY OF THE CHLOROPHYLL SYSTEM

Brown and Escombe (1905) made determinations of this value.

First of all we require to know the amount of energy necessary to convert 1 c.c. of carbon dioxide to hexose. This can be calculated from the heat of combustion of hexose, and amounts to 5·02 calories. To obtain the maximal efficiency, it is necessary to take account of the fact that the amount of light can be diminished nearly twelve times without affecting the rate of synthesis with the usual carbon dioxide tension of the atmosphere (Brown and Escombe, 1905, p. 86). Of the total radiation falling on the leaf, 65 to 78 per cent. is retained by it; the rest is transmitted or radiated to the surroundings. The greater part of that retained is used for purposes such as transpiration, that is, for evaporation of water. To find out how much is actually used for photo-synthesis, Brown and Escombe compared the amount absorbed by the white and green parts of a variegated leaf. In a particular case the white part absorbed 74·5 per cent. and the green, 78·7 per cent., so that 4·2 per cent. was absorbed by the chlorophyll. Now, it was found that, in *Tropæolum majus*, a total amount of incident light equal to 0·041 calorie per sq. cm. per minute caused the decomposition of 0·00034 c.c. of carbon dioxide per sq. cm. per minute. Since the energy stored in the conversion of 1 c.c. of carbon dioxide to sugar is 5·02 calories, that stored in 0·00034 c.c. is $0·00034 + 5·02 = 0·0017$ calorie per sq. cm. per minute. This is equal to 4·1 per cent. of the total incident radiation. We have just seen that 4·2 per cent. of the total incident radiation is absorbed by the chloroplasts, so that,

as Weigert points out (1911, p. 106), we obtain the astonishing efficiency of 98 per cent. But it must be remembered that the experiments were not made on the same leaf and also, according to Blackman, the position and distribution of the chlorophyll should be taken into account, which make the percentage of incident light absorbed by it 10 per cent. instead of 4·2 per cent. and the efficiency is reduced to 41 per cent. In any case, the maximum efficiency is a high one and is only obtained under exceptional conditions. It is usually about 20 per cent. A valuable critical summary of work done on chlorophyll is given by Jürgensen and Stiles (1917).

THE ACTION OF ULTRA-VIOLET LIGHT

The greater number of the constituents of living cells are colourless, that is, they do not absorb rays of the wave length of visible light. Many of them, however, absorb ultra-violet light so that it is not surprising to find that radiations of this kind have a very powerful effect on living cells as a rule.

The use of the absorbing power for ultra-violet of some constituents of living cells for the purpose of photographing them has been referred to above (page 9), as also the use of the fluorescence excited in them by ultra-violet light absorbed.

A series of important researches on ultra-violet light is at present being carried on by Victor Henri with several coadjutors, the results of some of which have been published (see Mme. V. Henri, Victor Henri, J. Larguier des Bancels, and R. Wurmser, 1912).

The first part of this work consisted in the determination of the wave lengths of the light emitted by various sources of ultra-violet light and of the absorption of screens. For the purpose of investigation it is clearly useful to have screens which will cut off ultra-violet and transmit visible light and others which will cut off the visible rays and transmit the ultra-violet rays. The most useful of the former was found to be "euphos" glass, which, in a thickness of 0·75 mm., cuts off very little of the visible spectrum, but only allows a very small amount of ultra-violet to pass. For the latter purpose, a colloidal solution of silver, prepared by the electrolytic method, is valuable. In a thickness of 20 mm., this allows no visible rays to pass, but is fairly transparent to ultra-violet, even waves as short as 219 $\mu\mu$ are slightly transmitted. In 10 mm. thickness, about 5 per cent. of the red, yellow, and green rays pass, but as much as 30 per cent. of the ultra-violet, in its middle region. Lehmann's (1910) modification of Wood's filter is much used. This consists of a double cell of Jena "uviol" glass, of 2 mm. thickness, that is, 6 mm. of the glass in all. The depth of each chamber is 5 mm. One is filled with saturated copper sulphate, the other with a solution of nitroso-dimethylaniline in a strength of one part in 12,000. The filament of an incandescent lamp is just visible through it, while it transmits a considerable amount of ultra-violet.

The absorption of egg- and serum-albumin was next studied. In the former the absorption is feeble for rays longer than 300 $\mu\mu$, increases to a maximum absorption band at 280 $\mu\mu$, has a minimum again at 250 $\mu\mu$, and then rises again; so that, for the extreme ultra-violet, the extinction coefficient exceeds 1,000. It may be noted that the mercury arc in a quartz tube sends out rays, of moderate intensity, as short as 220 $\mu\mu$, for which the absorption coefficient of albumin is over 1,000, and intense rays as far as 238 $\mu\mu$, for which the absorption coefficient is nearly 200, so that it is not surprising that protoplasm is very sensitive to the extreme ultra-violet of this lamp, as we shall see.

It has long been known that various small animals, such as those tiny Crustacea found in fresh water, flee from places illuminated by ultra-violet light, and Mme. V. Henri and Victor Henri (1912, pp. 12-21) have found that Cyclops is very useful for experiment. If it be illuminated for two to five minutes with the quartz mercury arc, it first shows great agitation, then becomes immobile. In this state, if kept in the dark, it remains for some hours motionless, but very sensitive to renewed illumination, to which it responds by a vigorous movement. It is thus easy to make exact measurements with it. After a day in water, it has become normal again.

A certain minimal duration of illumination is necessary for a reaction to

take place. This duration is less, the greater the proportion of ultra-violet in the light. Thus, through a quartz screen, the time is about two seconds; through 10 mm. of colloidal silver it is only increased to twenty-five seconds, although the ultra-violet is much diminished by the screen; through "euphos" glass, no reaction was produced in 200 seconds.

There is also a minimum intensity of illumination below which no reaction is obtained however long the exposure. There is also a certain intensity of illumination at which the necessary time of exposure is the shortest, an intensity greater or less than this requiring a longer time.

It is interesting to note that there is also a phenomenon of summation of sub-minimal stimuli, repeated at intervals, similar to that which we have seen in spinal reflexes.

A remarkable fact is that the excitability to ultra-violet rays is *independent of temperature*, a fact which shows that the exciting cause of the reflex movement is a photo-chemical reaction, whose products, no doubt, excite receptors of some kind in the outer surface of the animal. These receptors then excite nerve endings.

Fatigue can be produced to ultra-violet light in Cyclops by very short duration of radiation, if the peripheral receptors have been modified by prolonged previous radiation or by cocaine, but not if the anæsthesia is of central origin, by ether, for example. In this latter case, the peripheral organs are left intact. These facts show that the reaction studied is really due to photo-chemical changes at the periphery.

Since the absorbing power of protoplasm is very great, especially for the shorter wave lengths of ultra-violet, the effect can penetrate for only a short distance. In such small organisms as bacteria, however, it may affect the whole organism, so that the action, which is a lethal one in such cases, obeys the laws of photo-chemical reactions. In larger organisms, we have to take account of the diffusion of the products of photo-chemical change, and their action at places remote from that where they are formed. The laws are, therefore, more complex, and the effects last longer than the actual exposure.

The following table (from Victor Henri, etc., 1912, p. 33) gives the thickness of a layer of protoplasm which reduces ultra-violet light of different wave lengths to one-tenth its value, or absorbs nine-tenths of it.

Wave Length in $\mu\mu$.	Thickness of Protoplasm in μ.
240·5	79
238·5	58
231·3	18
226·5	9
219·5	6
214·4	3·8

Action on Bacteria, Tissues, etc.—An important practical question is that of the lethal action of ultra-violet light on micro-organisms, since it has been used for sterilisation of water. Equally important is its destructive effect on the tissues of higher animals, as used in the therapeutic method of Finsen.

Hertel (1905) has studied in some detail the effect of the magnesium line of 280 $\mu\mu$ on bacteria, protozoa, and some toxins, etc. Bacteria were found to be killed in from fifteen to sixty-five seconds. Mme. V. Henri and V. Henri (1912, pp. 29-31) find that there is no particular wave length which is specially lethal, but that the effect increases more and more the shorter the wave length, as far as tested, that is, up to 214 $\mu\mu$. Protozoa are killed in about the same time as bacteria, according to Hertel. The body swells, watery drops appear on the surface, and finally disintegration occurs. Rotifers, nematodes, and molluscs are also killed. On the tadpole, the effect is swelling of the epithelium, followed by migration of pigment and nuclear division, together with stasis of blood in the capillaries. On the cells of Elodea, slowing of the protoplasmic movement was noted, which was much less if illuminated by ordinary light at the same time. A longer exposure is required for tissue cells than for bacteria.

Diphtheria toxin was destroyed in five minutes, but the antitoxin was not destroyed in thirty minutes. Various enzymes were also rendered inactive.

Hertel regards the effect as due to *reduction* processes for three reasons:—

1. The effect is less in the green Hydra than in the colourless one, presumably due to the oxygen afforded by chlorophyll.

2. Oxyhæmoglobin is reduced.

3. If alizarin blue is injected into the veins of a rabbit, the brain is blue, but, if acted on by ultra-violet light, the dye is reduced to its colourless derivative.

An interesting point is that, if rays of equal energy (as measured by the thermopile) are taken, one of 440 $\mu\mu$, the other of 280 $\mu\mu$, Hertel found that rotifers are killed by the short waves in fifteen seconds, by the long waves only after four or five hours. This fact shows strikingly that it is not a question merely of energy, but of the actual wave length, no doubt because the short waves are absorbed by the protoplasm.

We have seen how large a number of photo-chemical reactions are brought about by ultra-violet light, so that we may expect to find it active also on protoplasmic systems. But the nature of these reactions in the latter case is still unexplained. One of the great difficulties in the therapeutic application of ultra-violet rays to stop the growth of malignant cells is the very rapid absorption by the superficial cells, so that the active rays do not penetrate more than a short distance. Considerable success has attended the treatment of superficial skin growth, such as lupus, by ultra-violet light. Since hæmoglobin has so active an absorption for ultra-violet (see especially the data of Victor Henri, etc., 1912), Finsen (1901, p. 70) uses a method of compressing the blood out of the area of tissue to be acted on by light.

The great effect of ultra-violet light on the skin is familiar to every one in the inflammation (erythema solare) called *sunburn*, which results in a brown coloration. It may be pointed out that this is not an effect of heat, in fact it is more liable to occur in cold surroundings, probably owing in part to the fact that the heat of the sun's rays is not noticed and no means taken to protect the skin from their action. It is, no doubt, due to the products of some photo-chemical reaction, acting on the arterioles, and it would be interesting to know whether, like the effect of oil of mustard, it is an axon reflex in sensory nerve fibres.

The Eye-Media.—Of course, if the eye is exposed to ultra-violet light, severe conjunctivitis is caused. Workers in this light have to wear spectacles impermeable to the short waves and frequently also to protect all parts of the skin exposed. This is especially so in electric welding, since the arc spectrum of iron is very rich in ultra-violet lines. E. K. Martin (1912) finds that the cornea absorbs all rays shorter than 295 $\mu\mu$. The lens is thus protected from the most active rays, although it is capable of absorbing rays between 300 and 400 $\mu\mu$, which might affect it and cause opacity (cataract), unless kept back by a screen outside. It was found, however, that while the mercury arc caused conjunctivitis, no change in the lens could be detected.

Hallwachs' Effect.—There is one further effect of ultra-violet light of theoretic interest in connection with the nature of photo-chemical change. Hallwachs (1888) noticed that a negatively charged insulated metal, such as a gold leaf electrometer, loses its charge when illuminated with ultra-violet light. This appears to be a case analogous to those in which light energy is stored, since the light energy is changed into the kinetic energy of moving electrons, shot from the metal.

PHOTO-DYNAMIC SENSITISATION

Although ultra-violet light has so much more action on protoplasm than visible light has, it has been found by several observers that light which has no action by itself on infusoria, bacteria, or blood produces the effect of ultra-violet light when certain dyestuffs are present. Although the dyes used were, for the most part, fluorescent, this does not seem to be an essential factor. For a complete account of the work, the reader is referred to the monograph by Tappeiner and Jodlbauer (1907). The general nature of the phenomenon will be clear from the few remarks following. Hertel (1905) exposed certain bacteria to light of 448 $\mu\mu$. This had no effect on them, either with or without the presence of eosin, 1 part in 1,200. Eosin has no absorption band in this position. Ultra-violet light of 280 $\mu\mu$ killed

them in sixty seconds, without eosin. A third experiment consisted in taking light of 518 $\mu\mu$, that is, in the position of the absorption band of eosin, and making it of about the same energy as that of the ultra-violet light previously used. Alone, this light had no effect, as would be expected, since it has a longer wave length than that found ineffective in the first experiment. On the other hand, in the presence of eosin, which has no action in itself, as the first experiment showed, the bacteria were killed in seventy to ninety seconds. It appears that the action of the eosin is to be compared to that of an optical sensitiser. The above results may be put in a table as follows:—

Wave Length in $\mu\mu$.	With Eosin.	Without Eosin.
518 (eosin absorption band)	Dead in seventy to ninety seconds	No change in half an hour
448	Nothing in half an hour . .	Do.
280	. . . .	Dead in one minute

Since chlorophyll is so active as an optical sensitiser, it has a very powerful "photo-dynamic" action.

The explanation of the phenomenon seems to be of the same nature as the similar one in the case of the photographic plate. The dye is adsorbed on the surface of the organisms and the effect is probably produced by an activation of oxygen, or perhaps by a product of the oxidation of the dye, since it requires the presence of oxygen for the phenomenon to occur.

An interesting experiment by Victor Henri, etc. (1912, p. 28), with colloidal selenium throws light on the question. The solution was fluorescent, but it had no effect in the dark, on certain protozoa. Under the ultra-microscope, it was seen to be a suspension of very minute particles. A certain number of the organisms took up these particles into vacuoles, where they aggregated into small masses. On exposure to light, it was found that only those organisms which had taken up the colloid were affected, thus showing the necessity of close contact and indicating a photo-chemical reaction, although not of the nature of the formation of a product which could act independently of the light. If this had been the case, those organisms which had not taken up the colloid would have been affected by the product diffusing from the others.

EFFECT OF LIGHT ON GROWTH

The lethal effect of light on bacteria was first described by Marshall Ward (1892), who did not recognise it as an effect of the ultra-violet rays.

There are other cases where light is known to have a retarding action on the growth of plants. Fungi, for example, grow more rapidly in the night than in the day time. The phenomena of heliotropic curvature, in its permanent stage, are due to diminution of the rate of growth in the places exposed to light. The first effect of light, however, as we have seen (page 126), is due to change of turgor, so that it is interesting to examine for a moment the effects of light on the *permeability* of the cell membrane. Tröndle (1910) showed that the result depends on the intensity of the light, a weak light causing diminution; a moderate one, increase; a very strong one, diminution again. These effects on permeability correspond to those on heliotropic curvature, so that the conclusion seems justified that we have to deal, in the primary stage of the latter, with changes in permeability. V. H. Blackman (1914) has investigated the action of light on the permeability of the excitable structures of the sensitive plant. The method used was that of the changes in its electrical conductivity. The interesting result was obtained, that during illumination an increased permeability existed, and that the first effect of cutting off the light was a further increase, after which the normal state returned. Whether this result has any relation to the similar one in the electric response of the retina cannot as yet be stated.

Tröndle holds that the effect of light on permeability is a complex one, depending on a photo-chemical change in the membrane, together with reactions on the part of the cell itself. Its use may be to facilitate the escape of assimilation products.

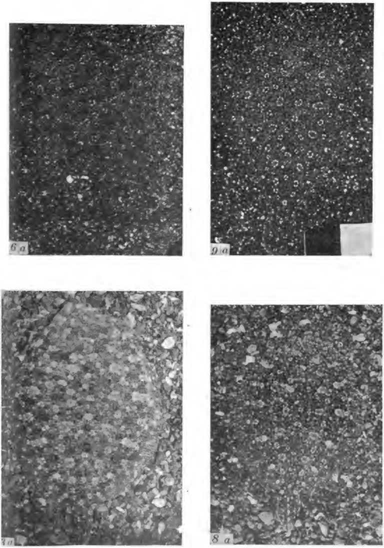


FIG. 181. CHROMATIC ADAPTATION.—Small flat-fish (*Rhomboidichthys podas*), photographed on ground of natural composition, gravel and sand of various degrees of coarseness. It is to be noted that the fish is completely uncovered, although appearing to lie under gravel, etc. (Sumner, 1911.)

(Reproduced by the kindness of Dr Sumner.)

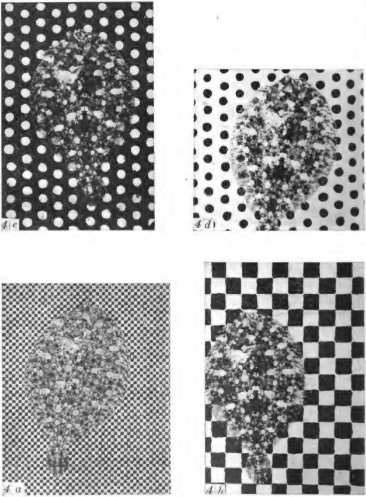


Fig. 182. Similar views to those of the preceding figure, on backgrounds of regular black and white patterns. The imitation of the smaller pattern is better than that of the larger one. In the latter cases, although there are large patches of black and white on the fish, their shapes do not very closely correspond with those of the background ; there is, however, a distinct indication of square or circular areas, respectively.

(Sumner, 1911.)

THE PHOTO-CHEMISTRY OF THE RETINA

It may be useful to refer back for a moment to the application of photo-chemical facts to the retinal process. There seems no doubt that the visual purple is an optical sensitiser; it is bleached by light as these are; and it absorbs nearly the whole length of the visible spectrum. Whether the products of its change are themselves capable of stimulating the light-receptors, or whether they act catalytically in bringing about other changes in these receptors, is unknown. It seems clear also that the peculiar form of the rods and cones must have some significance, but the difficulty of the problem is obvious, and in the present state of knowledge, speculation is of little use.

THE "CHROMATIC FUNCTION"

The power which certain animals have of modifying the colour of their skins to match that of their surroundings is well known. The case of the chameleon is often quoted. The manner in which it is effected is by the contraction or expansion of pigment cells of various colours in the skin. To this is sometimes added the iridescence due to interference of waves by means of a layer of fine crystalline structure. The work of Brücke (1851) on the chameleon was, after that of Pouchet (1848), the first experimental investigation of the question. Pouchet's work was on the frog and fish, and he introduced the name "chromatic function" to express the dependence of the adaptation on the nervous system, through the eye. Biedermann (1892) devoted much attention to the phenomena in the frog. The skin epithelium cells of this animal contain yellow granules and a deeper layer of crystalline particles, which show interference colours. In the subjacent corium, there are a set of black pigment cells. The sciatic nerve contains motor fibres for the latter cells and the chief centre appears to be in the optic lobes with subsidiary centres in the spinal cord. There is also a nerve supply to the chromatophores, by nerves accompanying the blood vessels. In the frog, the receptors for the reflex arc are in the skin, chiefly that of the discs of the toes. These are affected by the different qualities of the surfaces, which, in the habitual surroundings of the animal, are associated with definite colours, such as stone, grass, etc. When the toes are made anæsthetic, all colour adaptation disappears. The eyes play no part in the phenomenon. On the other hand, the adaptation to details, investigated by Sumner (1911) in *Rhomboidichthys podas*, a small flat-fish, allied to the turbot, are effected by means of eye receptors. The photographs in Figs. 181 and 182 will give an idea of the range of the adjustment. It is to be remembered that in reality the adaptation is more perfect than the monochrome reproduction indicates, since it took place to varying shades of brown, as well as to black and white. The change, when removed to a different background, was, in some cases, obvious after a few seconds. When the fish were made blind, the chromatophores went into their state of rest and no further adaptive reaction was possible.

The use of this mechanism to its possessor seems to be twofold. The animal is rendered invisible both to its enemies and also to the smaller fish which serve as its prey.

RADIO-ACTIVE PHENOMENA

Although the effects of radium and similar elements which give off charged particles, are not, strictly speaking, those of light, they may for convenience be mentioned here.

It was shown by Hardy that the negatively charged β-particles of radium produce coagulation of oppositely charged colloids, and the effect appears to be an electrical one.

The effects produced by radium on living tissues are very similar to those of intense ultra-violet light, but more powerful. They have been used in therapeutics for similar purposes. The explanation of their action is not yet clear. The advantage of radium as regards ease of application to the spot required is obvious. For further information on the extremely interesting phenomena of radio-activity,

the reader is referred to the books by Rutherford (1913) and by Soddy (1911, 1914), and, as regards its action on living tissues, to that by Finzi (1914).

X-RAYS

The X-rays of Röntgen, in their effect on cells, are also similar to those of ultra-violet light, but the destructive effect continues for a long time and does not appear at once in its full magnitude. The results of repeated exposure are very serious, since the rays penetrate to deep tissues and are able to produce degeneration of the seminiferous cells of the testis, for example. This fact of relatively slight absorption by tissues, other than bone or certain metallic salts, has made the use of X-rays very valuable in discovering the nature of displacement of bone, and so on. We have also seen their application to the study of the movements of the alimentary canal.

As to the nature of X-rays, the view is now generally held that they are similar to light waves, electro-magnetic, but of a very short wave length, 0·1 to 10 $\mu\mu$.

Further information may be obtained from the book by Kaye (1914).

PHOTOGRAPHY IN PHYSIOLOGY

The use of photographic methods of recording phenomena has been described above (page 460). We may add here the photography of absorption spectra. It is plain that plates sensitive to the whole of the visible spectrum are necessary. Wratten's "Panchromatic" will be found suitable, especially in the fine-grained variety known as "M" plates. These are prepared especially for the photography of objects under the microscope. The kind of negative usually required is not identical with that of landscape photography; for convenience of reproduction, a "hard negative" generally serves best. The developer used by Willstätter and Stoll (1913) will be found excellent for such purposes. It is made thus: Solution I., 500 c.c. distilled water, 50 g. crystallised sodium sulphite, 5 g. hydroquinone, 1 g. metol. Solution II., 500 c.c. distilled water, 50 g. potassium carbonate. For use, mix 30 c.c. of each with 60 c.c. of water (120 c.c. together) and develop for three minutes at 18° to 20° in darkness and fix in acid bath.

The methods of direct colour photography, such as Lumiére's "autochrome" process, have been used to prepare some beautiful photographs of stained microscopic preparations. Hartridge's safe-light (1915) is made on physiological principles.

SUMMARY

The dependence of life on the receipt of radiant energy from the sun makes it of importance to understand how this energy can be converted into forms useful to the organism without the necessity of passing through the state of heat, in which a considerable part of the free energy would be lost.

The manner in which this is done is by conversion directly into chemical energy by means of what are known as photo-chemical reactions.

These reactions are also of importance and interest with relation to the receptor organs for light impressions.

Light cannot act unless it is absorbed (Grotthus's law). The amount absorbed by a medium of various thicknesses is regulated by Lambert's law, which states that it is a logarithmic function of the thickness.

The "extinction coefficient" is the most useful form in which the absorption power of a particular solution is expressed. The extinction coefficient is the negative logarithm of the light which has passed unabsorbed through a thickness of 1 cm. It is different for different wave lengths. This is the form in which it is most convenient for practical use, but the original definition made it

he reciprocal of the thickness of the medium which sufficed to reduce light of a articular wave length to one-tenth of its value. The manner in which it is lerived from Lambert's and Beer's laws and its application to spectro-photometry re described in the text.

The phenomena of resonance play a large part in the mechanism of photo-hemical reactions. If the vibration period of a molecule coincides with that of ny of the light waves falling upon it, the molecule will be set into resonant gitation by means of the light energy absorbed. This vibration usually leads to hemical change. Luther's theory of the mechanism of the resonance process and ts consequence is given in the text.

Photo-chemical reactions do not obey the law of mass action, because their rate s controlled by the amount of light energy absorbed per unit time.

In order to set a photo-chemical reaction in train, a certain amount of light nergy is absorbed, so that the first stage is always associated with the taking up f energy. The later stages may be either similar to that of the chlorophyll ystem, in which the continuance of the reaction depends on a continual supply of ight energy and results in a storage of energy, or the reaction may be one which roceeds of itself with evolution of energy, but is accelerated by means of a atalyst produced by light. The catalyst may disappear in the reaction itself and equire to be formed by the continual action of light, as in the case of hydrogen nd chlorine. Or it may be more or less permanent and continue to act after the ight is removed. Another class of reactions is that of the coupled reactions, in which the products of the light reaction are used up at once in another reaction with loss of energy.

Optical sensitisers belong to the class of catalytic light reactions. A system, nsensitive to light of a particular wave length, can be made sensitive to it if a dye e present which absorbs this wave length. Since the light is absorbed by the ensitiser, it must act by means of the changes produced in this, resulting in the ormation of some substance, frequently active oxygen, or some catalyst, which cts on the system which is by itself insensitive to the particular light in question.

The Bunsen-Roscoe law states that the product of the intensity of the light nd its time of action produces a constant effect, so that intensity and time of ction can mutually make up for each other. This law is replaced by an ex-onential ratio when a photographic plate is developed after exposure. This fact eems to depend on the intervention of an adsorption phenomenon in the process f development.

The phenomena of fluorescence and phosphorescence are shown to be, probably, ases of photo-chemical reactions with storage of light energy, which is given off gain afterwards.

Chemi-luminescence is the name applied to the emission of light in a reaction t a temperature much below that corresponding to the wave length of the light iven off, if the system had merely been raised in temperature by the application f heat. It consists in the direct conversion of chemical energy into light, without assing through heat, and is the converse of those photo-chemical reactions in which ight energy is converted directly into chemical energy, as in the chlorophyll ystem of the green leaf.

The reactions brought about by the chloroplasts of the green leaf result in the torage of a large amount of energy derived from the sun. Carbon dioxide and water are changed into starch and oxygen.

Although the presence of the pigment chlorophyll is indispensable for the occurrence of the reaction, owing to its absorption of the light energy, there is no atisfactory evidence to show that carbon dioxide can be reduced by the pigment lone.

Chlorophyll is the ester of a complex acid, consisting of pyrrol derivatives inked to magnesium. This acid is combined with a monatomic hydrocarbon lcohol, phytol, with twenty carbon atoms.

There are two forms of chlorophyll in the leaf, one a product of oxidation of the other. These are accompanied by two yellow pigments, carotin and xanthophyll, unsaturated and autoxidisable hydrocarbons. These latter are not related to cholesterol.

The relation between chlorophyll and hæmoglobin is described in the text.

The absorption of light by chlorophyll is chiefly in the red and is in the position of the maximum energy of the solar spectrum during the greater part of the day.

The maximum action of chlorophyll is in light of a wave length corresponding to that of its absorption bands.

There is reason to suppose that formaldehyde is the first product of photo-synthesis. This is subsequently, perhaps also under the action of light, polymerised to higher carbohydrates.

Although a substance giving aldehyde reactions is split off from chlorophyll by the action of light in the presence of oxygen, there is no evidence that this substance is other than a decomposition product of the pigment itself, perhaps of the phytol constituent. Its production takes place in the absence of carbon dioxide.

Along with the production of an aldehyde by light and oxygen, a peroxide of some kind is formed, just as in the case of ordinary optical sensitisation by dye-stuffs. It is doubtful whether this is the source of the oxygen evolved in the normal course of the photo-synthetic process.

At the present time, therefore, we have no evidence that chlorophyll acts otherwise than as an optical sensitiser for the reactions going on in the complex system of the chloroplast. We have no information as to these complex reactions, except that possibly iron plays a part in them. It must be remembered, however, that the chemical structure of chlorophyll is a rather remarkable one, so that conclusions as to its behaviour must not be made too hastily.

Certain electrical changes have been noticed to occur in green leaves under the action of light. They appear to be connected with the photo-synthetic action, since they are absent unless carbon dioxide is present.

When the light available is deficient in the rays absorbed by chlorophyll, other optical sensitisers are found to be present and these absorb the rays which actually reach the cell.

A brief discussion of the factors affecting the rate of photo-synthesis is given in the text.

The efficiency of the photo-synthetic process is, at its optimum, a high one.

Many of the constituents of living cells absorb ultra-violet light to a considerable degree.

The reaction of certain small animal organisms to ultra-violet light obeys definite laws. A minimum duration is necessary; there is a limit of intensity below which no reaction occurs, and the effect is independent of temperature. These facts point to the occurrence of a photo-chemical reaction, whose products excite skin receptors

The direct action of ultra-violet light on micro-organisms and on tissue cells is a lethal or destructive one. The shorter the wave length, the more powerful the effect with equal energy of the radiation, probably owing to the greater absorption of the short waves by protoplasm.

Similar action can be produced by visible light in the presence of a dye which can absorb this light (photo-dynamic sensitisation).

Application of the facts of photo-chemical reactions to the retinal process is suggested in the text.

Light causes changes of permeability in the cell membrane and, in plants, has been shown to have the effect of retarding growth.

Certain animals adapt the colour and pattern of their skin markings to suit the background against which they are seen. In the frog, the receptors for the reflex are situated in the toes and the eyes play no part. In the fish, the eyes are the receptors.

The effects of radium and of X-rays on living tissues are similar to those of intense ultra-violet light. X-rays are generally regarded as being light waves of extremely short wave length.

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CHAPTER XX

OXIDATION AND REDUCTION

In the preceding chapter we have seen how, by the aid of light energy, the chemically stable system of $CO_2 + H_2O$ is converted into one of higher potential energy, carbohydrate and oxygen. In reconversion to its original state, the energy of this system is utilised by living organisms for various purposes. But, although the system possesses considerable potential energy, it is, chemically, a stable one. This is, indeed, necessary, in order that its energy should not be given off spontaneously at all times, but only when required. Molecular oxygen is unable to oxidise carbohydrate, except at an extremely slow rate; although there are certain substances, such as simple aldehydes and those compounds called "unsaturated," which are "autoxidisable," that is, capable of oxidation by molecular oxygen. In this process, moreover, by a mechanism which will require discussion later, other substances, not themselves oxidisable by molecular oxygen, undergo simultaneous oxidation; we have a "*coupled reaction.*"

This mechanism alone, however, will not satisfy the requirements of the case. We have, accordingly, a catalytic mechanism in addition, which has the effect itself of "*activating*" oxygen.

It may be remarked here that the processes of oxidation and reduction are not merely of use for the purposes of obtaining energy by complete combustion. Intermediate stages result in the formation of substances required for use in chemical reactions of importance for other purposes. The monograph by Dakin (1912) will serve to show the numerous cases of interest in this respect.

In the discussion of the question it must not be forgotten that the oxidation of one substance is always accompanied by the reduction of another. As Hardy points out (note appended to Drury's paper, 1914, p. 175), the place where oxidation takes place in a cell may also be a reduction place, if a different zero of oxidation potential be taken. A convenient one is that of atmospheric oxygen. A region of such a chemical potential would be a reduction place for compounds whose oxygen potential is higher than that of atmospheric oxygen, but an oxidation place for substances in which it is less than that of atmospheric oxygen. The absence of agreement as to the zero may lead to confusion.

ACTIVE OXYGEN

The first question to be investigated is the nature of the state into which oxygen is put, so as to be able to oxidise substances upon which it has no action in its ordinary molecular state.

It is sometimes stated that it is in the "atomic" state, but this suggestion does not really help much, since we do not actually know what the difference between the atomic and molecular state is.

Again, the active state is sometimes spoken of as the "nascent" condition. It is a matter of experience that chemical elements or groupings are more ready to enter into combination at the moment of their liberation from previous combination. It appears that, in the process, chemical energy is made use of before it has become degraded into heat, hence more free energy is available.

The most probable view seems to be that it is in the process of changing its valency, or electric charge, that oxygen is in the active state. At all events,

electrical phenomena are associated with the activation of oxygen, as shown by the following facts.

Oxidation and reduction do not always mean the addition or removal of oxygen or hydrogen. The change from ferric to ferrous salts is a reduction, but consists in the conversion of trivalent iron to bivalent iron.

Haber (1898) has shown that the reducing action of hydrogen, developed on an electrode, depends on the electrical potential there. In this way, a reduction process can be carried to a particular point and no further. Thus, nitro-benzene can be reduced to azoxy-benzene and no further, if the cathode potential is low. Dony-Hénault (1900) also showed that alcohol can be quantitatively oxidised as far as aldehyde, with a proper anode potential.

The phenomena connected with the autoxidation of phosphorus and its effect in condensing a steam jet were referred to above (page 31). We may note that the effect consists in the production of "gas ions" and is not shown by the products of the reaction, but only by some process taking place in the actual course of the reaction itself.

Ostwald (1890, p. 76) suggested that reduction means a diminution of charge, that is, a loss of (negative) electrons.

Oxygen, as we know, may be bi- or quadrivalent. In the peroxides it is probably the latter, and, when split off in a particular way, it has unusually powerful oxidising properties, a fact of importance in physiological oxidations. Its activity appears to depend on its readiness to give up its extra charges.

AUTOXIDATION

The theory of the process which takes place in the spontaneous oxidation of phosphorus, benzaldehyde, or other such substances, was suggested by Bach (1897), and by Engler and Wild (1897), independently, and was adopted by Ostwald (1900, 1). It has been observed as an experimental fact that, in such reactions, there are formed simultaneously two oxides in equivalent proportion, a lower oxide and a peroxide. Now, in the production of the former, energy is given out, whereas the latter has a higher oxidation potential than the oxygen gas, and requires energy to form it. This energy is derived from that afforded by the production of the simple oxide.

We saw, in speaking of "coupled reactions," that these reactions, in which energy afforded by one reaction is used to enable another to take place, must be capable of being expressed as parts of one and the same complete reaction; we see, then, why there is always a quantitative, equivalent relation between the simple oxide and the higher oxide. Schönbein (see the monograph by Engler and Weissberg, 1904, p. 9) was the first to point out that half the oxygen is used to oxidise the substance itself, while the other half is "activated." The peroxide obtained in the oxidation of phosphorus is ozone and its formation should be described thus. In the process there is first formed a peroxide of phosphorus, which then splits up into ozone, on the one hand, and a lower oxide of phosphorus on the other hand. Thus:—

$$mP + nO_2 = P_mO_{2n} \qquad (1)$$

$$\underset{\text{(intermed. oxide)}}{P_mO_{2n}} = \underset{\text{(lower oxide)}}{P_mO_{(2n-3r)}} + \underset{\text{(higher oxide)}}{rO_3} \qquad (2)$$

Whether this intermediate oxide is to be detected or not depends on the rate of its decomposition.

The way in which the above reaction is described is that of Ostwald. It certainly is in agreement with experimental facts and explains why the two products of an autoxidation are always in equivalent proportion, since they were at one time combined in one substance. Ostwald also points out that it is a general rule that the most unstable product of a reaction makes its appearance first and is then decomposed. But if the peroxide is formed first, it is difficult to see how the energy is obtained for the process.

It is interesting to call to mind also what Larmor has pointed out (1908). If we consider the great distance between the molecules of a gas as compared with their own dimensions, it is easy to see that an impact between molecules takes place only at a comparatively rare frequency. Suppose that the molecules are of

two different kinds, capable of reacting together; the impacts will, in a certain proportion of cases, be between different kinds of molecules, and some of them will give rise to combination. If there is also a third kind of molecule present, the compound molecule formed by the first two will occasionally meet with this third kind, and combination of three kinds of molecules result. But the chances are almost infinity to one against three different kinds of molecules meeting simultaneously in such a way as to combine together. We are, no doubt, justified in extending these considerations to substances in solution. It appears, therefore, to be extremely improbable that a reaction between three different molecules ever occurs in one stage, or, indeed, a reaction between two of one kind and one of another kind, or between three of one kind. All reactions should, if possible, be represented as taking place in stages between two molecules only at a time. Nernst (1913, pp. 475 and 595) calls attention to the same fact with reference to the probability that reactions of a higher order than bimolecular must be very rare. In any case, their velocity must be very small. Reactions which appear to be trimolecular are often found, on investigation, to take place in two bimolecular stages (see also van't Hoff's Lectures, 1901, Heft 1, p. 196).

Mrs Onslow (1920) has obtained evidence that in plants there are catalysts which accelerate autoxidation, especially that of catechol derivatives. By this means the peroxides required for the next process are quickly produced.

PEROXIDES AND THEIR CATALYSTS

The peroxide, ozone, which is produced in such autoxidations as that of phosphorus, has a powerful oxidation potential, so that, for example, it liberates iodine from potassium iodide with great rapidity. Now, the peroxides which we find produced in living cells have an oxidation potential which is not so high as this; they consist either of hydrogen peroxide, or have a similar constitution. Their appearance in the photo-chemical reactions of the green leaf has been met with in Chapter XIX. In fact, in the presence of water, the organic peroxides of the latter type readily form hydrogen peroxide.

Peroxides of this type only liberate iodine from potassium iodide very slowly, and their power of oxidising such substances as sugar is practically nil. We have, however, already seen an example of a typical catalytic process, with formation of an intermediate compound, in the increased action of hydrogen peroxide on hydriodic acid when minute amounts of molybdic acid are added (Brode). We note that the molybdic acid is found at the end unchanged.

There are other substances, such as ferrous iron in the well-known Fenton's reaction (1894), which act as catalysts on hydrogen peroxide with the separation of what we may, for convenience, continue to call "active" oxygen. Moreover, from various animal and plant tissues, enzymes have been prepared which have the same effect. These have been called by Bach and Chodat (1903) "*peroxidases*."

The nature and properties of the numerous substances concerned with physiological oxidations and reproductions have led to much work and caused much difficulty in the interpretation of the complex phenomena observed. A consistent and intelligible theory was first proposed by Bach and Chodat (1904), to whom we owe the greater part of the accurate investigation of the subject (see the article by Bach, 1913). In the following pages I describe the phenomena on the basis of this theory, although further research may make necessary some modification in it.

The intervention of cell structures will come up for discussion in a later paragraph.

There is an enzyme, to which we have already referred, called *catalase*, which has the property of decomposing hydrogen peroxide without activating the oxygen given off. The result of its action is molecular oxygen merely, given off as gas. Although catalase is of very common occurrence in plants and animals, the part it plays is, at present, somewhat uncertain. In any case, it does not directly concern us here, since it does not bring about oxidation, although, according to Bach (1913, p. 182), it plays an important part in protecting sensitive parts of the cell mechanism from the easily diffusible hydrogen peroxide, formed in the oxidative processes. Bach states, also, that in a mixture of hydrogen peroxide with both catalase and peroxidase, part of the peroxide is decomposed with production of active oxygen,

the other part into molecular oxygen, according to the relative amount of the two enzymes present. Catalase might thus act as a *regulator* of the oxidation process.

Peroxides are characterised by the presence of two atoms of oxygen, directly united together. If we take oxygen as quadrivalent, such a substance as hydrogen peroxide contains two atoms of oxygen united by three valencies, and when one atom is split off in the active form, this atom possesses four free valencies to be satisfied by combination with an oxidisable substance. Or we may put it thus, in the formation of the peroxide, the valency of the oxygen is changed from two to four. A substance gives none of the reactions of a peroxide, however many oxygen atoms it may contain, unless some are directly connected together. Persulphuric acid, $HSO_4 - O_4SH$, thus behaves as a peroxide, similarly other per-acids and their salts. Since the atoms of molecular oxygen are united together, it seems that, when it combines with an autoxidisable substance, the primary product must be a peroxide, as is assumed in the equation given above (page 581). At the same time, ordinary oxygen has no peroxide properties, although by the addition of another atom to make ozone, we obtain a powerful peroxide. In a peroxide, therefore, the two atoms directly united must, apparently, be themselves united to some other atom or group, which may be oxygen itself. The meaning of this is not clear. These groups may, indeed, be either "electro-negative," as in persulphuric acid, or "electro-positive," as in sodium peroxide, $NaO - ONa$.

Most peroxides are hydrolysed by water in two stages, thus:—

$$A\left\langle\begin{array}{l} O \\ | \\ O \end{array}\right. + \begin{array}{l} H \\ | \\ OH \end{array} \longrightarrow A\left\langle\begin{array}{l} OH \\ O-OH \end{array}\right. \qquad \text{- - - - - (1)}$$

$$A\left\langle\begin{array}{l} OH \\ O-OH \end{array}\right. \longrightarrow AO + HOOH \qquad \text{- - - - - (2)}$$

Therefore, a peroxide, arising by autoxidation of an oxidisable substance produced by a cell, gives rise as a rule to the formation of hydrogen peroxide.

Catalytic Activation of Peroxides.—Indigo blue is very slowly oxidised in air, presumably with the usual production of a peroxide. Oil of turpentine is oxidised in a similar manner at a considerable rate. In the presence of the latter, the oxygen of its peroxide is transferred to the indigo, which thus undergoes a rapid oxidation.

Now Bach holds (1913, p. 148) that the oil of turpentine should be called a catalyst in this reaction, since it does not appear as a constituent of the oxidised indigo, although it is not itself in its original form at the end of the reaction. I think, however, that it tends to confusion to speak of a catalytic process, where energy for a reaction is afforded by the agent called catalyst, as in this case, and that it is better to call it a coupled reaction. At the same time, it must be confessed that it is difficult to draw a very marked line of demarcation between this kind of reaction and that of the acceleration of the action of hydrogen peroxide on hydriodic acid by molybdic acid. The only essential difference is that in the latter, true catalytic action, the catalyst is recovered as in the beginning. This must be considered to be the real criterion, for even when the catalyst is not recovered intact, the change it has undergone is independent of the main reaction, merely incidental, whereas the oxidation of the oil of turpentine is an essential part of the reaction.

The cases where colloidal platinum and related metals act as catalysts are regarded by Bach (1913, p. 149) as precisely similar to that of indigo and oil of turpentine, since a peroxide like that of oil of turpentine is supposed to be formed, but, in this case, becoming metal again. It seems to me, however, that these phenomena of heterogeneous catalysis are not, as yet, satisfactorily explained by the hypothetical assumption of various oxides of the metal, for whose actual existence there is not sufficient evidence. There is the fundamental difference, also, that the energy needed to raise the oxidation potential of the system is not afforded by chemical degradation of the platinum, as it is in the case of oil of turpentine and similar cases of autoxidation. The bearing of this question on the nature of Bach's "oxygenase" will be seen presently.

The process of autoxidation results, then, in the production of a peroxide and in the simultaneous oxidation of certain other substances present in the system,

which are not, by themselves, accessible to molecular oxygen. In the presence of water we generally find that the peroxides form hydrogen peroxide, which has not a high oxidation potential. But it was known already to Schönbein (1860) that ferrous salts, in extremely small amount, strongly accelerate the action of hydrogen peroxide on oxidisable substances. Ferrous salts, in fact, as also those of copper and of manganese, accelerate the oxidising power of oil of turpentine, benzaldehyde, etc., no doubt by action on the peroxides produced. It is supposed by some that these metallic salts combine with the peroxides to form unstable "complexes," which split off the peroxide oxygen more readily than the original peroxides, like the permolybdic acids of Brode's experiments. In any case, the metal reappears in its original form and is thus a true catalyst.

PEROXIDASES

When we come to apply the above phenomena to the process of oxidation in living cells, we find that the problem is by no means easy. The oxidation systems met with are often of great complexity and the enzymes unstable. The result has been that, although we are in possession of a large number of facts, their relation to a general theory is not a simple matter to make out.

The reader will find an excellent account of the subject in the monograph by Kastle (1910); we must confine ourselves here to those facts which seem to give most guidance in the formation of a general theory. When we refer to certain preparations as coming from this or that plant or animal organ, it is not to be supposed that similar substances are not of general occurrence. It happens that, for various reasons, particular enzymes and so on are more readily isolated from their admixture with other substances in some cases than in others.

The gum-resin, *guaiacum*, happens to be a convenient test for the presence of active oxygen, since one of its constituents, guaiaconic acid, is oxidised to a blue substance by active oxygen, but not by ordinary oxygen, nor even by such peroxides as that of hydrogen. It is best used in the form of a solution of guaiaconic acid in dilute alcohol, and must be freshly made.

Suppose that we take a scraping from the surface of a potato, some fresh blood fibrin, or various other products of living cells, and apply a drop of guaiaconic acid solution. A blue colour is produced, showing the presence of active oxygen. But this simple experiment does not lead us far in the analysis of the mechanism.

Now, Bach and Chodat (1903) showed that from the root of the horse-radish a solution could be prepared which did not give the blue reaction spoken of, neither did it give off oxygen gas when hydrogen peroxide was added. But if to this solution we add hydrogen peroxide, guaiacum is oxidised with the production of the blue colour. The solution must therefore contain something which activates hydrogen peroxide. This constituent is destroyed by heat, precipitated by alcohol, and shows the general properties of an enzyme, and was therefore called "*peroxidase*."

We are next naturally led to look for evidence of the presence of hydrogen peroxide, or similar peroxide, together with peroxidase, in those cases in which guaiacum is blued without the necessity of adding hydrogen peroxide. An experiment by Bach (1914, p. 225) is of interest here. Fresh potato juice oxidises tyrosine rapidly. If acted on by alcohol, a precipitate is formed which has scarcely any action on tyrosine, unless hydrogen peroxide is added. Hence hydrogen peroxide can take the place of a similar substance naturally present.

Now, when we take a solution of peroxidase and add guaiacum and hydrogen peroxide, we naturally obtain the blue colour whether free oxygen is present or not, since the hydrogen peroxide supplies what is required. But, suppose we take potato scrapings, put them into a tube through which we lead hydrogen or coal gas, until the oxygen is displaced, and then, by means of a tap funnel, previously fitted, we drop guaiaconic acid on to the potato, we see that no oxidation takes place until air is allowed to enter the tube. If we consider this result for a moment, we shall see that its meaning must be this: Peroxidase is present as usual, but the absence of active oxygen, unless air is present, shows that no peroxide is available for the peroxidase to act upon. The peroxide is therefore

formed, when air is admitted, in the process of the taking up of oxygen by autoxidation of a spontaneously oxidising substance.

This system of autoxidisable substance, peroxide and peroxidase, is that which was at one time thought to be itself an enzyme and called an "*oxidase.*" The actual enzyme concerned is peroxidase, as was shown by Bach and Chodat (1904); the other constituent, which forms a peroxide in presence of oxygen, is called by them, "oxygenase." Oxidase has thus two components, each inactive alone.

It may be noticed that the termination "ase" implies enzyme nature, but we have seen reason for regarding the production of peroxides in autoxidation as not being of the nature of catalysis. It does not seem to me, moreover, that the easier destruction by heat of the oxygenase than the peroxidase proves its enzyme nature. In this respect, I am in agreement with Moore and Whitley (1909), but it is, after all, only a question of the meaning of the word "catalyst." Otherwise the scheme of Bach satisfies the facts excellently. Mrs Onslow gives the name "oxygenase" to the catalyst which accelerates autoxidation and is not identical with the oxidisable substance.

We may summarise the matter thus:—the substrate, which is to be subjected to oxidation, is usually one that, by itself, takes up oxygen by autoxidation so slowly as to be imperceptible. This is accelerated by the presence of a genuine autoxidisable substance (Bach's "oxygenase"), which acts in a way similar to that of oil of turpentine and such readily oxidised substances. This action is produced by the formation of peroxides. These peroxides are rendered still more active by the enzyme, peroxidase, which accelerates the transfer of oxygen to the substrate by splitting it off from the peroxide. The value of the enzymic last part of the process may be this. We saw that the oxidation of an autoxidisable substance may effect the oxidation of other substances present during the reaction itself, so that it necessitates the presence of both free oxygen and an easily oxidised substance. The peroxide formed in this reaction, on the other hand, may very well persist for an appreciable time and be thus available for the bringing about of an oxidation when acted on by peroxidase coming into play as required.

We have seen above that salts of iron, manganese, or copper act on peroxides in the same way as the enzyme, peroxidase, and Bertrand thought that his "laccase" owed its activity to the presence of manganese. It was found later that iron could take the place of manganese, and Bach (1910) states that he has prepared "oxidases" free from both these metals; whether any other metal, acting similarly, was present is not stated. The hypothesis that peroxidases are particularly active forms of one of these metals is, at present, the one most in agreement with experimental facts.

The metals found to be active as "peroxidases" are those capable of existing in two states of different valency.

By the addition of one of these metallic salts to a system of peroxide and peroxidase, a mixed system can be produced, with increase of activity.

Further, Dony-Hénault (1908) has prepared what he calls an "artificial laccase" in the following way. A solution is taken containing, in 50 c.c. of water, 1 g. manganese formate, 0·4 g. sodium bicarbonate, and 10 g. gum arabic. This is precipitated by alcohol. The precipitate is redissolved in water and again thrown down by alcohol. This substance is of interest for two reasons:—1. As an obvious adsorption compound, but yet precipitated unchanged by alcohol, no doubt because the precipitation is practically *total*. 2. As an enzyme made artificially. It is precipitated by alcohol. But the more important property is that it acts catalytically. It does not seem to be destroyed by heat, but a similar substance made from albumin and manganese by Trillat (1904) is destroyed by boiling, while it has been stated that natural laccase is not so destroyed. The property clearly depends on the nature of the emulsoid colloid in association with the metal.

The natural "oxidase" systems are more or less "*specific*": each appears to act on a special group of substrates, or even on one only. There is reason to suppose that this depends on the particular organic peroxide constituent of the complex system, rather than on the peroxidase. This "specificity" is not unknown in inorganic catalysts, thus Wolff (1908) found that colloidal ferrous ferrocyanide acts as a peroxidase towards phenols, but does not accelerate the action of hydrogen

peroxide on hydriodic acid. The direct action of the substrate on the enzyme, chemically destructive or by alteration of its physical properties, has always to be taken into account in these specific relations.

It may be asked, what is the function of the gum in our artificial laccase? Before we can answer this question, we must examine into the state of the metallic salt in solution, whether it be iron, copper, or manganese.

Salts of all these metals, especially in the dilute solutions in question, are hydrolysed in water. In other words, the solutions used consist of hydroxides of the metals in the colloidal state. This seems to be their active state, although it is not easy to say why the colloidal hydroxide should be more active than the ion. The experiments of Moore and Webster (1913) on the formation of formaldehyde by ultra-violet light, in the presence of colloidal ferric hydroxide, may be called to mind. Bertrand also (1897) tested the oxidising effect of a series of manganese salts on hydroquinone and found those to be the most powerful which were the most hydrolysed in solution.

If, then, the colloidal state is of so much importance, it seems clear that the activity must be in direct relationship to the extent of the surface. Hence the use of gum, albumin, and so on. The way these "stable" colloids act in protecting a suspensoid colloid, such as ferric hydroxide, from precipitation by electrolytes, thus ensuring a high degree of dispersion, has been explained above (page 97).

A peroxidase is, then, in all probability, a peculiarly active form of the colloidal hydroxide of manganese, iron, or copper, preserved in this active state by the presence of an emulsoid colloid, such as gum or albumin. It is to this stable colloid that the enzyme owes its precipitation by heat or by alcohol, and, possibly, any degree of specificity that it possesses. A view essentially the same as this was suggested by Perrin (1905, p. 103).

ENZYMES CONCERNED WITH REDUCTION

Although it has long been known that fresh animal and plant tissues have the power of reducing nitrates to nitrites, and it was held by some that the process is a catalytic one, the existence of *reducing enzymes*, analogous to the oxidising ones, has not been generally accepted.

Schardinger (1902), however, made the observation that fresh milk rapidly reduces methylene blue, indigo, and so on, if an aldehyde, such as formic or acetic aldehyde, be present; whereas it has no such action in the absence of the aldehyde. The property is abolished by boiling, and has been used as a test to distinguish fresh from sterilised milk. It was clearly proved by Trommsdorff (1909) that this reaction is due to an enzyme and not to bacteria. If microbes are present, the milk, after some hours, acquires the property of reducing methylene blue without the addition of an aldehyde.

Now it is clear that we must have a formation of nascent or active hydrogen, and that it must come from the water in the system. If a reaction is going on which takes up oxygen from water, hydrogen will be set free. It is probable, then, that we have to do with a reaction of the kind called by Bach (1913, p. 150) "*hydrolytic oxidative-reducing reactions.*"

A reaction of this kind has been already described (page 266) in the oxidation of a-amino-acids to aldehydes, as discovered by Strecker. To understand the mechanism, however, a simpler system is better, and we may take that of the decomposition of water by hypophosphites in the presence of metallic palladium, as investigated by Bach (1909).

To begin with, it must be admitted that the mechanism of such reactions is by no means clear as yet and I confess to a certain amount of misgiving as to the chemical nature of the intermediate compounds supposed to be formed. The system is a heterogeneous one, palladium and similar metals being insoluble, and the phenomena of adsorption or surface condensation, always present in such systems, should be kept in mind. In the following description when oxides of platinum, etc., are spoken of, it is very doubtful whether they really have the definite chemical formulæ assigned to them, since they have not been isolated. Similarly, it may be remembered that the permolybdic acids formed in Brode's typical case (page 324) are said to be a series of this kind:—

$$MoO_3.1/2H_2O_2,\ MoO_3.H_2O_2,\ MoO_3.3/2H_2O_2,\ MoO_3.2H_2O_2.$$

The formulæ rather suggest adsorption compounds. We may also call to mind the controversy between Faraday and de la Rive (see page 306 above).

To return to the ***hypophosphite system***, hypophosphites do not undergo oxidation in water alone at any measurable rate. But in the presence of finely divided palladium, this takes place. Bach puts it thus: the water is decomposed and its HO is used for oxidation of the hypophosphite, the hydrogen is taken up temporarily by palladium, and then set free. Thus:—

$$\begin{matrix} H \\ O \end{matrix}\!\!>\!P\!<\!\!\begin{matrix} H \\ OH \end{matrix} + \begin{matrix} OHH \\ OHH \end{matrix} = \begin{matrix} H \\ O \end{matrix}\!\!>\!P\!<\!\!\begin{matrix} OH \\ OH \end{matrix} + H_2 + H_2O.$$

Palladium acts as a true catalyst; minute amounts decompose indefinite amounts of hypophosphite. If an easily reducible substance is present, the nascent hydrogen reduces it.

If we take an aldehyde in place of hypophosphite, we find that the presence of metals of the platinum group does not accelerate to any great degree the decomposition of water. A further addition is required in the form of an easily reducible substance as "acceptor" for the nascent hydrogen as it is formed; such substances are methylene blue, indigo, nitrates, and so on. None of these are reduced at any perceptible rate by formaldehyde alone without platinum. In the reaction, the aldehyde is oxidised to the corresponding carboxylic acid. The case of methylene blue is instructive because this dye contains no oxygen, so that the additional atom of oxygen required to convert formaldehyde into formic acid must come from the water by some means.

The explanation of this fact suggested by Bach (1911, 1) is, shortly, as follows. Water may be looked upon as an unsaturated compound, $H_2O=$, since oxygen is quadrivalent, at all events potentially. Since $H^{\cdot}$ and OH' ions are also present in water, as we have seen, it seems probable that "unstable complexes" may be formed thus:—

$$\begin{matrix} H \\ H \end{matrix}\!\!>\!O\!<\!\!\begin{matrix} H^{\cdot} \\ H^{\cdot} \end{matrix} \quad \text{and} \quad \begin{matrix} HO' \\ HO' \end{matrix}\!\!>\!O\!<\!\!\begin{matrix} H \\ H \end{matrix}$$

The first may be called "hydrogen suboxide" or "oxygen perhydride," analogous to the metallic salts M_4O, such as Ag_4O. The second is the hydrate of hydrogen peroxide. We have seen reason in Chapters VII. and VIII. to hold that ions are associated with water molecules.

The acceleration by platinum of the oxidation of aldehydes to form acids can be explained on the view of Engler and Wöhler (1901) that colloidal platinum combines with molecular oxygen to form a peroxide, PtO_2, which, in its turn, reacts with water to form the hydrate—

$$\begin{matrix} HO-Pt-O=O \\ \quad\quad\quad\;\; | \\ \quad\quad\quad\;\; H \end{matrix}$$

This substance may also be supposed to be formed by reaction of platinum with the $H_2O(OH')_2$ present in the water. It acts as a powerful oxidising agent on formaldehyde. Since $H_2O(OH')_2$ is used up, the equilibrium is disturbed, more is formed, and so the catalytic process continues.

If we admit the presence in water of H_4O, it is not unlikely that platinum metals should form strongly reducing hydrides by combination with this.

The two complexes of $H^{\cdot}$ and OH' ions with water are only present in very small concentration, so that unless one of them is used up, say the hydride in reducing methylene blue, the oxidation of the aldehyde can only take place to a very small extent.

I have already remarked that this view assumes the existence of chemical compounds of rather doubtful nature, and Bach himself states (1913, p. 154) that the question requires further investigation to make the mechanism clear. The view given certainly explains the occurrence of active hydrogen, which is otherwise difficult to account for.

We naturally turn next to look for the evidence of an enzyme, in milk and tissue cells, which plays a part similar to that of the platinum metals. Since a peroxidase causes the activation of peroxide oxygen, we may call, with Bach (1913, p. 161), an enzyme which causes the activation of perhydride hydrogen a "*perhydridase*."

The enzyme shown by Schardinger and Trommsdorff to be responsible for the reduction by fresh milk of methylene blue, if an aldehyde be also present, has been referred to above. Now, reducing action of fresh tissues has been described by

various observers and ascribed to "reductases" or "*reducases*." In analogy with the oxidase system, as

peroxidase + peroxide

we may regard a reducase as

Schardinger's enzyme + aldehyde.

Schardinger's reaction appears then to be a hydrolytic-oxidative-reduction process, in which the aldehyde is oxidised by the oxygen of water, while the hydrogen thus set free reduces the methylene blue to its leuco-base, by the intermediation of the enzyme.

Liver tissue, ground up with water, has been known for a long time to be capable of reducing methylene blue at a rapid rate. Bach (1911-1912) investigated this reaction from the above point of view. Is there an enzyme present like that in milk and an oxidisable substance acting like aldehyde?

If liver is rubbed up with five times its weight of 2 per cent. sodium fluoride and filtered through linen, the emulsion, diluted five times, has very little action by itself on methylene blue. But, if a small quantity of acetic aldehyde be added, the reduction is rapid. If previously boiled, the emulsion has lost this property. Filtration through paper deprives it of the enzyme, which remains on the paper. But a solution can be made by extracting the liver with 1 per cent. sodium bicarbonate. This is filtered through linen, the filtrate neutralised exactly with acetic acid and again filtered through paper. Alcohol produces a precipitate in the filtrate, and, from this precipitate, the enzyme can be extracted by half per cent. sodium bicarbonate. The solution reduces methylene blue in the presence of aldehyde. The enzyme is very unstable. A similar preparation can be made from lung, spleen, kidney, or thymus.

This enzyme also reduces nitrates to nitrites in the presence of an aldehyde, just as fresh milk does, so that it is not of a specific nature.

The nature of the substance which takes the place of aldehyde in the cell system is not yet known. It appears to be insoluble, since it is left with the cell débris on the linen in Bach's process, as given above.

An oxidase (or phenolase) produces its oxidation by the aid of free oxygen, while the reducase acts by means of the combined oxygen in water, indirectly. Thus:—

oxidase = peroxidase + oxidisable substance (oxygenase) + oxygen

reducase = Schardinger's enzyme + oxidisable substance (oxygenase) + water.

In the first case, we may say that free oxygen is reduced, but the reduction process stops here and is covered by the oxidation process; in the second case, hydrogen is set free by the decomposition of the oxidising agent, water, and further reduction processes are set in progress.

Cannizzaro's Reaction.—This reaction consists in the simultaneous oxidation and reduction of aldehydes, by which the hydrogen of water converts one molecule to the alcohol and the oxygen converts the other one to the acid:—

$$2R.CHO + 2HOH = R.CH_2OH + R.COOH + H_2O.$$

Parnas (1910, 2) showed that liver tissue greatly accelerates this reaction, a result which is obviously an aspect of the action of Bach's perhydridase.

The enzyme in extracts of organs which oxidise salicylic aldehyde appears to be similar and the reaction to be a case of acceleration of Cannizzaro's reaction.

Plant Reducases.—There is a perhydridase in potato juice and other plant extracts (Bach, 1913, 2). This requires for its action the presence of one of the lower aldehydes and then reduces nitrates to nitrites, but apparently methylene blue is not attacked.

The increased consumption of oxygen by acetone yeast in the presence of methylene blue, as described by Meyerhof (1912, 3), seems to be a related phenomenon. The experiments of Palladin, Hubbenet, and Korsakov (1911) on the higher plants also bear upon the question. Methylene blue causes greater oxidation in etiolated plants and the presence of oxygen is necessary for the effect in the bean plant, although not in the pea; a fact which appears to be connected

with the anaerobic production of alcohol in the latter. In the presence of oxygen, the dye does not suffer reduction.

THE GUAIACUM REACTION OF BLOOD

Hæmoglobin has the power of oxidising guaiaconic acid. This has been shown by Buckmaster (1907) to be due to the iron contained in it; the iron-free derivatives do not give the reaction.

We have seen reason for regarding iron as a peroxidase, but since a peroxidase requires a peroxide to act upon, it appears that, in the blood reaction, there must be some substance present which is autoxidisable.

It is interesting to note that lecithin is oxidised in air by ferrous-ammonium sulphate (Thunberg, 1911). It would thus form a peroxide, like benzaldehyde does. Certain other cell constituents, nuclein, albumin, glucose, oleic acid, are not oxidisable by iron alone.

TYROSINASE

The production of pigments by the action of an oxidising enzyme on tyrosine has been referred to above (page 359). This enzyme appears to be of frequent occurrence, not only in fungi, where it was first found, but also in animal tissues, amongst others in insect larvæ. According to Bach (1914), the system called tyrosinase is a complex one. The effect of one of its constituents is to *reduce* the tyrosine. The products are then easily oxidised by the oxidase also present. The behaviour is similar to that of the respiratory pigments of plants, described by Palladin (1909), which are alternately oxidised and reduced by the agency of enzymes.

THE OXIDATION SYSTEM OF THE CELL

So far as we have arrived, the chemical process of oxidation in the cell seems to be as follows. Some autoxidisable substance in the cell takes up molecular oxygen, with the formation of peroxides and activation of half of the oxygen. The other half of the oxygen serves for *complete* oxidation of part of the autoxidisable substance. These peroxides are acted upon by peroxidase, with further increase of active oxygen, which is able to bring about oxidation of substances not autoxidisable and otherwise difficult of oxidation. But when we come to apply the facts learnt by study of extracts or of disintegrated cells to the interpretation of phenomena taking place in the living cell, we find that there is something else to be taken account of. This we may call "structure," meaning thereby not merely the coarse structure seen under the microscope, which is probably less important than the ultra-microscopic structure, of colloidal nature, to which attention was called previously (page 19).

The suggestion made by Warburg (1914), as to the purpose of the energy set free by oxidation in cells which do no external work, has been referred to in an earlier chapter of this book (page 32).

We have already met with cases in which the importance of structure forces itself upon the attention. The non-disappearance of lactic acid in muscle, after rubbing with sand (Fletcher and Hopkins, 1907), the inability of Harden and Maclean (1911) to obtain press juices from tissues which could continue to consume oxygen, the great effect on oxygen consumption of alkalies which do not enter the cell itself (Warburg, 1910), and other similar actions on the surface, may be mentioned.

It should be pointed out that it is impossible to draw a hard and fast line between the phenomena to be considered here and those of tissue respiration, to be dealt with in the following chapter, but I will attempt not to repeat statements more than is necessary.

The observations of Warburg and Meyerhof (1912) serve to illustrate the problem before us. The red blood corpuscles of birds contain nuclei and, in their normal condition, consume oxygen in considerable amount. If a press juice is

made by Buchner's method, no oxygen consumption is to be detected. Similarly, mechanical disintegration puts an end to the process. Now, although yeast juice, as is well known, is still able to cause alcoholic fermentation, Warburg and Meyerhof have shown that the activity of yeast cells in this respect is greatly diminished by rubbing with sand. Similar observations on other cells were made by Battelli and Stern, Palladin and others, to which reference will be found in the article by Warburg (1914).

Now cells can be killed by such treatment as dehydration with acetone, etc., without obvious destruction of structure; in fact, ordinary microscopic structure is intact. Warburg points out (1914, p. 317) that acetone and ether make a good fixing method for cells even of the delicacy of the eggs of the sea urchin in division. The important point in the process, as used for the investigations with which we are now concerned, seems to be the rapid drying. The chemical composition is practically unchanged, even the lipoids remain in the cells. The effect of this treatment on yeast cells is greatly to diminish their fermentative power (Buchner and Hahn, 1903, pp. 87 and 269). On bacteria (staphylococci), Warburg and Meyerhof found that its effect on oxygen consumption and carbon dioxide production was not to abolish them completely, although they were greatly diminished. Under favourable conditions, this respiratory process might remain constant for some hours, with a respiratory quotient of 0·65 to 0·9. The injury to the oxidation process was, in fact, less than to the fermentative power of yeast by similar treatment. Of course, in such experiments, it is essential to know that all the cells were "killed," that is, incapable of growth. To do this, after drying with acetone, they were heated to 100°; and shown to be sterile after the experiment was concluded. If treated with acetone alone, without heating, the oxygen consumption falls only to one-third of the normal, although cultures showed that nearly all the cells were killed. In absolute amount, the oxygen consumption of such completely sterile cells does not fall much below that of some other normal surviving cells, those of the liver consuming 2·7 c.c. of oxygen per gram per hour, while the sterile staphylococci consumed 1·5 c.c.

If we compare these results with those on the eggs of the sea urchin, we find some instructive facts. The unfertilised eggs, rubbed with sand, show at first a nearly normal oxygen consumption; this slowly decreases, so that in the third hour it is only one-quarter to one-third of the normal. Fertilised eggs, already divided, have, along with their more developed organisation, a greater consumption of oxygen than unfertilised ones. Moreover, on rubbing with sand, the decrease is greater, so that in the *first* hour it only amounts to one-quarter to one-third of the normal. Acetone-dried unfertilised eggs also have a measurable oxygen consumption, although it is less than when they are simply rubbed with sand.

In order to obtain some further idea as to what is to be understood by cell structure, Warburg (1914, p. 315) calls attention to the fact that in the muscle cell a much larger proportion of the chemical energy appears as free energy, useful for doing work, than if the cell is disintegrated; in the latter case the chemical energy obtained from oxidation processes is all degraded to heat. By cell structure, then, we mean those elements with which, or by whose aid, the work of the cell is carried on. They are arrangements by which the chemical energy of the oxidation processes is caught, as it were, before it has fallen to the state of heat. If we look upon the cell constituents as chemical compounds merely, without the assistance of some mechanism, nothing but heat could be obtained on oxidation. The same thing applies to a petrol motor with its fuel. If smashed up and mixed together, nothing but heat would be obtained by burning the mass.

If we divide up a cell nucleus into a thousand particles and consider them distributed throughout the cell, the nuclear structure is destroyed, as shown by the fact that the ordered movement shown in karyokinesis is no longer possible.

As remarked above, the *cell membrane* is to be regarded as a very important element of the cell structure or mechanism.

We see then that the oxidations effected by the aid of the enzyme mechanisms, treated of in the earlier parts of the present chapter, take place in the cell in the

presence of a mechanism which makes use of their energy in the actual progress of the reaction.

Further than this it is scarcely possible to go in the present state of knowledge. A few further facts, nevertheless, are of interest.

Permeability.—Although acetone produces no visible change in the eggs of the sea urchin, it renders the cell membrane permeable to electrolytes. A living egg placed in distilled water rapidly bursts, after swelling up. Acetone eggs, after soaking in sea water, undergo no change of volume in distilled water.

Effect of Nucleus.—Although blood corpuscles containing nuclei consume more oxygen than non-nucleated ones, the fact does not necessarily imply that it is the nucleus alone that is responsible. There is more protoplasmic material in the former kind of cells. Certain evidence, also, shows that fragments of protoplasm, free from nuclei, consume oxygen. The nucleus, in fact, counts as a part of the cell structure. If red blood corpuscles of birds be frozen and thawed, cytolysis occurs, the membrane is ruptured and certain cell constituents escape. It has been shown by Warburg (1914, p. 322) that some of the structural parts are not disintegrated, but, being insoluble, can be centrifuged off. By this process, we obtain an upper layer of structureless cell substance and a lower one of structural elements. When separated, oxygen consumption is nearly absent from the upper layer, but by the lower layer it is absorbed in about the same amount as by the original mixture. See also p. 613 with regard to blood corpuscles.

Effect of Increase of Structural Differentiation.—The comparison of the oxygen consumption of the fertilised egg of the sea urchin with that of the unfertilised egg gave Warburg (1908 and 1910) opportunity to study this factor. The increase is considerable, but not directly proportional to the number of new nuclei formed. The change from one nucleus to a thousand, for example, only causes a threefold increase in oxygen consumption. This fact indicates that the "structure" in question is not the visible one of nuclei, and so on. A remarkable fact, however, is that, if the eggs are cytolysed by placing in distilled water, and shaking, the resulting suspension of apparently structureless débris consumed as much oxygen as the normal cells in the case of the unfertilised eggs; but it was reduced to one-tenth in the fertilised, dividing eggs, although the structure did not appear to be so completely destroyed as in the former case. A significant fact, which shows that the oxidation process, even in the unfertilised eggs, was not normal after cytolysis, is that the carbon dioxide production ceased.

Effect of the Cell Membrane.—We have already referred to the fact that changes of permeability occur in the act of fertilisation, and Warburg (1908) has shown that, coincidently with this, the rate of oxygen consumption rises considerably. Further, it was shown, as already mentioned (page 142), that alkali, even when it does not enter the cell, causes a large rise in the oxygen consumption. It is evident, then, that changes in the cell surface alone produce profound effects on the cell mechanism, and further evidence is afforded that the "structures" are of very minute character, since no obvious change takes place in the cell. The minute nature of the protoplasmic elements was pointed out above (page 19). The machinery can be put out of work, although no visible change may have occurred. As if, in a petrol motor, the accumulator cells used for ignition were discharged.

Lillie states (1913) that the formation of indo-phenol blue by oxidation of a-naphthol and dimethyl-paradiamino-benzene takes place most rapidly at the nuclear and cell membranes of the frog's blood corpuscles. The passage of induction shocks is said to accelerate this reaction, so that electrical polarisation of these surfaces is held to play a part.

Effect of Cyanide.—The action of potassium cyanide in extremely small concentration is to stop all oxidation processes in cells, without doing any permanent damage. Recovery can be obtained by washing away the cyanide. This paralysis of oxidation has been referred to above (page 448) in relation to the analysis of the muscle processes. The work of Weizsäcker (1912) on the heart of the frog gives some interesting facts. We find, to take an example from his table on p. 140, that a particular heart, in absence of cyanide, performed 1,180 g.-cm. of work with a consumption of oxygen corresponding to 23 mm. of

the scale of Barcroft's apparatus, and an evolution of 25 mm. of carbon dioxide. In the presence of m/6,000 potassium cyanide, the same heart performed more work (1,380 g. cm.) with the consumption of only 8 mm. of oxygen, and gave off 9 mm. of carbon dioxide. On p. 143, it is shown that the excitability of the heart to electrical stimuli, even when the consumption of oxygen has been completely abolished by m/2,000 cyanide, is unchanged.

The explanation given by Warburg of the action of cyanide will be found immediately.

Relation to Catalysts.—Warburg and Meyerhof (see Warburg, 1914, p. 334) have obtained results with unfertilised, cytolysed sea urchin eggs which show the importance of iron. The ash of the eggs was found to contain considerable amounts of iron. The addition of iron salts to the egg substance caused very considerable increase in the oxygen consumption, an effect not produced by other metallic salts, not even by manganese.

Alcohol extracts were also made, evaporated to dryness, and the residue extracted with ether. A part remained undissolved, and this part consumed no oxygen, even on the addition of iron salt. The ether extract was evaporated, and the residue suspended in water. This suspension, by itself, consumed no oxygen, but, on addition of iron, the oxidation amounted to as much as that of the original egg substance. Substances of a "lipoid" nature are, therefore, responsible for the phenomenon.

Now, Thunberg (1911) has observed that lecithin, in the presence of iron, consumes oxygen at a considerable rate; and lecithin is present in the eggs. Further, we have seen reason, in preceding pages of this chapter, to hold that the activity of the peroxidases of the cell depends on their content in iron (or manganese). But, since lecithin is only slowly autoxidisable, its oxidation would need to be accelerated by Mrs Onslow's "oxygenase." A further difficulty is the absence of carbon dioxide production, both in the cytolysed eggs and in the action of iron salts on lecithin.

According to information given me by Dr Weizsäcker, Warburg has recently found that the amount of potassium cyanide required to stop oxidation in the egg cells of the sea urchin is precisely equal to that required to combine with the iron which they contain. This fact distinctly points to the iron as the catalyst concerned in oxidation. It is difficult, however, to see exactly what compound of iron, containing cyanide ion, could be reversible under the conditions of the life of the cell. It must be a complex ion of the nature of the ferrocyanic ion; but it is not a simple process to recover the iron from such compounds under conditions which would be possible in a protoplasmic system.

Vernon (1914, p. 220) points out that the indophenol oxidase is inhibited by narcotics in a series corresponding to their anæsthetic action on tadpoles, and draws the conclusion that the oxidation process is connected with lipoids. The fact certainly shows that surfaces play a part. Nucleo-proteins are shown not to do so

Mode of Action of "Structure."—Warburg (1914, p. 337) suggests that the essential importance of structure consists in the presence of surfaces for condensation of the catalysts active in the cell processes; in other words, for adsorption. The action of narcotics of the alcohol series is much greater on the fermentative power of yeast cells than on that of the press juice, apparently because these substances are highly adsorbed and drive off the enzymes from their state of condensation on the surfaces (see the paper by Warburg, 1913, 1, p. 20).

This observer does not think that the view of separate "reaction-chambers," although it may apply to other chemical reactions in the cell, is applicable to the case of oxidation, because the fluid contents presumably contained in these spaces can be changed by diffusion in certain cases, without affecting the oxidation processes. It does not appear to me, however, that the evidence is sufficiently convincing to show that the essential contents were actually changed in these instances. The active contents of the hypothetical, ultra-microscopic vacuoles might be indiffusible, or held by adsorption. Probably both surface condensation and microscopic reaction-chambers play a part.

There are some further experiments by Warburg (1913, 2), which require

mention here. Mammalian liver was rubbed with sand, water added, and the mixture centrifuged for ten minutes. A suspension of fine particles was obtained, which absorbed oxygen and gave off carbon dioxide, thus confirming certain statements of Battelli and Stern with respect to "water-soluble respiration." The amount of oxygen consumed was about one-fifth of that consumed by the intact liver in an equal time. The remaining four-fifths are what Battelli and Stern call the "chief respiration" (1909). The particles were small enough to show Brownian movements, but were removed by filtration through a Berkefeld filter. No doubt they would be removed by the Buchner method in Harden and Maclean's work. The Berkefeld filtrate certainly showed a slight oxygen consumption, about one-twenty-fifth of that of the entire liver, but there might have been a few ultra-microscopic particles present.

ENERGETICS OF OXIDATION IN CELLS

As frequently pointed out already, the energy required for the various purposes of the organism is derived, except in very special cases, entirely from oxidation. The necessity of considerable combustion in muscle cells doing external work is clear, as also where gland cells are performing osmotic work. But, as Warburg points out (1914, pp. 256-258), the oxygen consumption of nucleated blood corpuscles, of the central nervous system and of the developing egg, is difficult to understand, since there is no apparent work done, with the exception of a minimal amount. This is especially noticeable in the last case referred to, since the rate of oxidation has no relation even to the morphological changes taking place. What then becomes of the energy? It would seem wasteful if it were merely degraded to heat. Warburg, therefore, makes the suggestion already referred to, that there may be work done in a way that is invisible, but yet indispensable. It may be required to maintain the "structure" of the cell, in the sense of preventing the mixing of constituents by diffusion, in maintaining intact certain properties of the semipermeable membranes, such as electric charge, or possibly irreciprocal permeability and other states of which we have, at present, little knowledge.

The relation of oxygen to the work of the muscle cell has been given in some detail above (page 446), and reference also made to the work of secretion and so on. In the present place, some interesting investigations, chiefly by Meyerhof, on the total energy changes of certain isolated cells, as indicated by heat production, may be referred to.

Experiments on whole organisms, such as those done by Rubner, Benedict, Macdonald, etc., show that the heat production is practically identical with the loss of chemical energy of the food-stuffs. Bohr and Hasselbalch (1903) determined the heat production of the developing chick, comparing it with the respiratory exchange, and found it to be identical with that of fat, as indicated by the respiratory quotient. The fact is interesting as showing that the formation of the morphological structures which contain nitrogen uses up no measurable amount of energy.

The experiments of Meyerhof (1911) were concerned with the developing eggs of the sea urchin. The "caloric quotient" was first determined. This is the number expressing the amount of heat formed, in gram-calories, per milligram of oxygen consumed. Previous workers, Zuntz, Pflüger, Rubner, found this number to be, neglecting the second decimal place, when protein is burnt, 3·2; when fat, 3·3; when carbohydrate, 3·4 to 3·5. Now that of the developing egg is between 2·55 and 2·9. This number is made slightly lower if the heat of solution of carbon dioxide and that of its combination to sodium bicarbonate is taken into account. This value, moreover, remains the same, whether fertilised or unfertilised eggs are taken, or if cell division is prevented by the presence of phenyl-urethane, as in Warburg's experiments. If work had been done in formation of coarse morphological structures, it is plain that the values could not be the same in these different cases.

It will be remembered that Warburg showed that ammonia, which enters the cells, stops cell division, but increases slightly the oxygen consumption. Now

in such cases, Meyerhof found the caloric quotient raised to 3·3. If the heat of combination of ammonia with carbon dioxide be deducted, the value falls to 2·95; but this is the maximum deduction permissible, so that the value is certainly raised above the normal one.

As to the meaning of the low value of the caloric quotient, no evidence of the presence of carbohydrate was to be found, and there was no breakdown of protein. Fat, on the other hand, was found in sufficient amount to cover the heat produced.

Further experiments (1912, 1) were made with nucleated blood corpuscles of birds. In this case, a "normal" caloric quotient of a value between that of protein and fat was found. As Meyerhof remarks (1912, 2, p. 1), it can scarcely be an accidental coincidence that, when the normal caloric quotient was found, the cells were in a stationary condition, in the other case in active multiplication.

In the case of aerobic bacteria, Meyerhof (1912, 2) finds that the caloric quotient is from 4·5 to 4·7, whether growth is in progress or inhibited by deficiency of food material, being rather higher in the latter case. This high quotient seems to be due to reactions in the nutrient solution brought about by products of bacterial action.

Horace Brown (1914, p. 223) finds that the heat production of yeast, as grown in fermenting solutions, is, weight for weight, somewhere about seventy times as great as that of a man at rest. An explanation of this relatively enormous metabolism is suggested on the basis of the abnormal conditions under which yeast is cultivated for industrial purposes, as compared with its natural habitat on the outer skin of fruits. It must be remembered that we know now that there is no inverse proportion between fermentation and growth. In absence of oxygen, no growth takes place, but, as Pasteur showed, the fermentation process goes on with vigour. The cells remain constant in mass and in composition, so that no energy is needed for growth, yet, as Horace Brown puts it (p. 224), "there is an enormous activity in the metabolic mill, through which continues to pass an amount of substance which may amount to several times the mass of the cell in a few hours."

Further considerations on the question of anaerobic existence will be found in the next chapter.

In the paper referred to (p. 226), Horace Brown states that he has obtained evidence that less heat is evolved from the same amount of sugar fermented, when growth is taking place, than in its absence. Quantitative measurements of this kind would give, of course, what might be called the "heat of formation" of yeast.

THE OXIDATION POTENTIAL OF CELLS IN THE ORGANISM

The experiments of Ehrlich (1885) are of much interest in this respect, although they are not, in all cases, easy to interpret. Two dyestuffs were used in intravenous injection, both capable of oxidation and reduction. One of them, alizarin blue, is reduced with difficulty; it requires boiling with caustic alkali and glucose. The other, indophenol blue, is more easily reduced.

We saw in our first chapter that living protoplasm itself does not stain with soluble dyes; the two dyes used in Ehrlich's experiments were, accordingly, introduced in the form of suspensions, or colloidal solutions, and the particles were then taken up by the cells of various organs and found therein subsequently, either as the reduced, colourless derivative, or the oxidised, blue one, according to the oxidation potential of the cell system.

Alizarin Blue.—This dye does not become reduced in the blood, but is reduced in the liver, the renal cortex, the Harderian gland, and the lungs. The amount taken up depends, as would be expected, on the permeability of the cell membrane.

Indophenol Blue.—The heart and the brain, together with certain voluntary muscles, such as the diaphragm and the eye muscles, are blue. Some secreting glands also do not reduce the dye. By all other organs it is reduced.

In general, it may be said (Ehrlich, 1885, p. 109) that the reducing power of protoplasm lies between that required by alizarin blue and by indophenol blue.

But it is to be remembered that "protoplasm," as used here, means the cell constituents as a whole.

The organs may be divided into three groups:—

1. Those of high "oxygen saturation," in which indophenol blue is not reduced. Such are the grey matter of the brain, the heart, and some other muscular organs.

2. Those which reduce indophenol blue, but not alizarin blue. Such are the greater number of the tissues, smooth muscle, most voluntary muscles, and secreting glands.

3. Those which reduce even alizarin blue. Lungs, liver, fatty tissue, Harderian gland.

As to the meaning of the facts, Ehrlich points out that, in activity, as also in asphyxia, practically all cells become highly reducing, and that the state of a cell at any given moment depends on the rate at which it consumes oxygen in relation to that at which it is supplied. At the same time, it is necessary to assume that a series of substances of different reducing power make their appearance. Thus, a substance which has less affinity for oxygen than alizarin blue has cannot reduce it, and a further stage of reduction must occur before this takes place.

The important question of the facility of access of oxygen belongs to those to be discussed in the next chapter.

The fact that fat tissue has so high a reducing power, shows that oxygen avidity is not necessarily to be ascribed to great functional capacity. The chemical nature of certain permanent constituents of the cell must be taken into account. This should be kept in mind with regard to the paradoxical experimental fact of the reducing power of the lung tissue. Ehrlich ascribes the property to the stroma cells, not to the alveolar epithelium, and suggests that it may be due to an appropriate relative impermeability of these cells to oxygen, so that they shall not unnecessarily retard the aeration of the blood.

THE PRODUCTION OF LIGHT

After what we have learnt in the present and preceding chapters in connection with the phenomena of autoxidation and their relation to catalysts, together with that of chemi-luminescence, further brief reference may profitably be made to the problem of the emission of light by living organisms.

One of the most important practical questions at the present time is that of the improvement of the efficiency of our methods of artificial illumination. The majority of these, as used now, depend on the emission of light by substances when heated to a very high temperature. The higher they can be heated, the greater the proportion of light to heat rays. Hence the advantage of the metallic filament lamps over the old carbon filament, and especially that of the electric arc over other forms of illuminant.

We have seen, however (page 557), that, in the phenomena of chemi-luminescence, we have light emitted at temperatures far below those to which it would be necessary to heat a metallic wire in order to obtain light of the same wave length. We may put it thus, chemical energy is transformed directly to light energy, without passing through the state of heat.

Now the problem appears to have been solved by numerous organisms, although the quantity of light they emit is not great. Amongst these organisms we may mention fungi (including bacteria), protozoa, medusæ, insects, molluscs, and fish. For individual details of these organisms, the reader is referred to the articles by Mangold (1910), Dubois (1903, 1913), Coblentz (1912), and for plants, Molisch (1904).

The fact that living cells can emit light shows at once that the fact is not due to their temperature, as in the case of the ordinary sources of light. Phosphorescence, in the true sense, requires previous exposure to light, and is easily excluded. We are left then with phenomena related to chemi-luminescence.

Spectral examination shows, accordingly, that the light is limited to the middle region of the spectrum, usually having its maximum in the green (Langley

and Very, 1890). Fig. 183 gives photographs of the spectrum of the light of the fire-fly, taken by Coblentz. It will be seen that the spectrum extends considerably less, both towards the red and towards the violet, than that of the carbon filament lamp. It is, in fact, according to Coblentz, very like that of an ideal radiator at a temperature of 5,000°. Langley and Very (1890) showed that the heat radiation of *Pyrophorus noctilucus* (a South American beetle) is only one-four-hundredth of that given out by incandescent sources, such as candle light, reduced to the same luminosity.

In its chemical aspect, the phenomenon is evidently an oxidation. In many cases, the cessation of light in absence of oxygen and its reappearance on admission of oxygen have been demonstrated. The nature of the substance oxidised is not yet known. The particular case of Pholas has been referred to above (page 362). According to Dubois (1913), the oxidation here is under the control of an enzyme, an "oxidase"; the secretion is luminous after removal from the cells, as can readily be verified. In any case, the reaction seems to be independent of the "vital" activity of cells. Schultze (1864) finds a substance which reduces osmic acid in the luminous organs of the glow-worm, probably a derivative of oleic acid.

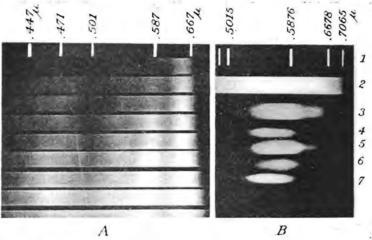


FIG. 183. LIGHT OF FIRE-FLY COMPARED WITH THAT OF ARTIFICIAL SOURCES.

A, Photographs of spectrum of carbon glow-lamp, for different exposures.

B, Photographed spectra of
1, helium vacuum tube.
2, carbon glow-lamp, four watts per candle power.
3, 5, and 6, fire-fly, *Photinus pyralis*
4 and 7, fire-fly, *Photinus pennsylvanica.*

The colours of the bright lines in the spectrum at the top are as follows:—
·447 μ, blue. ·471 μ, bluish green. ·5015 μ, green. ·5876 μ, yellow. ·6678 μ, reddish orange. ·7065 μ, red.

Note that the light emitted by the fire-fly is confined to the middle region of the spectrum and is most intense in the yellow-green, while that of the carbon glow-lamp is most intense in the orange (see the uppermost spectrum in *A*, below the bright line comparison spectrum). (Coblentz, 1912.)

(Carnegie Institution of Washington.)

There is evidence that a certain small amount of heat is developed in the active glands of the fire-fly (Coblentz, 1912, p. 33), but this appears to be associated with the secretory process.

Water is necessary. Dried material can, however, be brought to shine again by moistening.

Although the production of light can occur by reaction with oxygen of

substances formed in the cell, in many cases the process takes place already inside the cell itself. In other cases the luminosity does not make its appearance until the secretion is extruded.

According to Dubois, there are two substances concerned, an enzyme, "luciferase," and an oxidisable compound, "luciferin." Newton Harvey (1915-1920), in an extensive series of investigations on various organisms, has added much to our knowledge. Luciferin is thermostable and diffusible. It is oxidised in presence of oxygen by luciferase, which behaves as a specific peroxidase, to oxyluciferin, light being given off in the process. It was impossible to detect any evolution of carbon dioxide or production of heat. Potassium cyanide does not prevent the production of light. The reaction is regarded as being similar to the oxidation of leuco-methylene-blue, in which hydrogen is removed to form water. The oxidation-product in neither case can be reduced by mere exposure to zero tension of oxygen. A greater reduction-potential is required, such as that of the reducase system of milk or yeast. It is a remarkable fact that light itself reduces oxyluciferin. Thus, the reaction, inclusive of light energy, is a reversible one:—

$$\text{Luciferin } (LH_2) + O \rightleftarrows \text{oxyluciferin } (L) + H_2O + \text{light}.$$

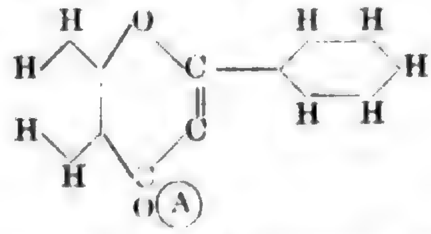
An extraordinarily small amount of the active substance is needed to give perceptible light.

A third substance, "photophelein," introduces experimental difficulties. It behaves like saponin in driving off luciferase from an inactive adsorbed state. Hence, when added to a mixture of luciferin with such luciferase, light is produced.

The use of the production of light to the organisms themselves is somewhat problematical. It may serve to attract prey and, in some cases, it appears to assist the progression of the organism in the dark, like an ordinary lantern.

THE COLOURS OF FLOWERS

In connection with chlorophyll and with oxidation enzymes, the nature of the coloured pigments of flowers is of interest. As Willstatter points out, it is a suggestive fact that the vitally important leaf pigment is the same in all plants, whereas those of the flower are of various chemical constitution. Muriel Wheldale (1911, 1913) showed that the pigment of the snapdragon is related to the flavones. This was confirmed by Willstätter and Everest (1913), and extended to the pigments of other flowers. Flavone is:—



This quinonoid structure, as is well known, is associated with coloured substances.

In the flower pigments, the oxygen atom marked (A) is replaced by hydrogen, while a varying number of the benzene hydrogens are oxidised or replaced by other groups. In the salts, the oxygen atom at the top of the ring becomes quadrivalent.

In the flower, the flavone derivatives are combined with glucose to form glucosides and are capable of oxidation under the influence of an oxidase. Thus Keeble, Armstrong, and Jones (1913) found that the yellow sap pigment of the wallflower is a mixture of hydroxy-flavone glucosides. These are readily hydrolysed by mineral acids, or, more slowly, by emulsin. The hydrolysed product, if reduced and again oxidised, gives a red pigment.

An interesting fact is the occurrence of the colourless chromogen (reduced pigment) in the flower, together with both an oxidising and a reducing enzyme. The former is active in water, inactive in alcohol. The latter is inactive in water, active in alcohol. Or perhaps this reducing enzyme is overpowered in water by the opposite action of the oxidase. The colour thus disappears in alcohol, returns in water. In our discussion of the synthetic action of enzymes we saw reason to believe that there are mechanisms in the cell capable of

reducing the effective concentration of water, and we see here that the removal of water retards oxidation, and thus facilitates the action of reducing enzymes.

The Mendelian factors are discussed in Miss Wheldale's book (1916).

SUMMARY

There are some substances which are oxidised by the oxygen of the air. Others, and these are the food-stuffs of most physiological importance, require the oxygen to be made "active," as it is often called. In other words, the system which causes the oxidation of such substances as sugar must have a higher oxidation potential than molecular oxygen has.

It seems to be most in accordance with experimental facts to suppose that it is when in the process of changing its valency, or electric charge, that oxygen is "active." This conception is practically the same as that of the nascent state. In the course of another reaction oxidative effects are frequently obtained, such as oxygen alone is unable to bring about. Chemical energy is thus made use of before it has become degraded to heat.

When a substance undergoes "autoxidation" by molecular oxygen, there are two oxides formed in equivalent proportion. In the production of the lower oxide, energy is given out, and this energy is utilised to build up the other oxide, which is a peroxide, and has a higher oxidation potential than the original system. The reaction is what is known as a "coupled reaction."

There is reason to believe that reactions between three or more different molecules at the same time rarely, if ever, take place. Probably all reactions occur in stages between two molecules at a time.

Those peroxides produced in the autoxidation of phosphorus and in some other cases, have a considerably higher oxidation potential than such peroxides as that of hydrogen. In the presence of water, as in the living cell, peroxides of the former type, if produced, react with water to form hydrogen peroxide.

Now such a peroxide as that of hydrogen has not sufficient oxidation power to bring about the combustion of glucose, for example. But there are inorganic catalysts, such as iron, and also enzymes, called "peroxidases," which decompose hydrogen peroxide with the liberation of "active" oxygen. These latter enzymes are found in the living cell.

Since the oxidations brought about by cells do not occur in the absence of oxygen although a peroxidase is present, we must conclude that the peroxide is absent. Hence, the peroxide is formed by the action of molecular oxygen on some autoxidisable substance in the cell.

In the actual process of autoxidation, another substance, itself difficult of oxidation, may be drawn into the reaction, as it were, and become oxidised. But the peroxides also formed in the process are acted on by the peroxidase of the cell, with formation of additional "active" oxygen.

Artificial oxidation systems, similar to the natural ones, "oxidases," can be made by the association of colloidal hydroxides of iron or manganese with an emulsoid colloid. The function of the latter appears to be that of maintaining the active constituent in a state of high dispersion and protecting it from aggregation by electrolytes.

Living tissues also produce reducing systems, which require the presence of substances such as aldehydes in order to show their activity.

In these reduction processes, the reactions called "hydrolytic-oxidative-reducing" have to be taken into account. The explanation of these reactions, as given by Bach, will be found in the text. They seem to consist in the decomposition of water and the formation of "unstable complexes" of hydrogen and hydroxyl ions with water molecules. The former acts as oxygen perhydride, the latter is the hydrate of hydrogen peroxide.

The perhydride is apparently decomposed by an enzyme, "perhydridase," analogous to peroxidase, with the activation of hydrogen. An enzyme of this nature has been prepared from liver.

In the living cell, the presence of autoxidisable substances, together with peroxidase, is not in itself sufficient to bring about the oxidations which actually occur. Disintegration of the cell, as by rubbing with sand, nearly puts an end to the consumption of oxygen by it, although the cell constituents are all present as chemical compounds.

These constituents must be organised into some kind of a mechanism or structure. This is not the ordinary structure visible under the microscope, since the latter may be unaltered, but the power of consuming oxygen absent.

There are some facts which show that a certain degree of oxygen consumption may remain after disintegration of the morphological structures by rubbing with sand.

The properties of the cell membrane are of importance for the oxidative processes in the cell.

The importance of "structure," no doubt, consists partly in the provision of surfaces for adsorption and activation by concentration of the catalysts concerned in the cell processes. Probably the maintenance of ultra-microscopic "reaction-chambers," by provision of semipermeable membranes, also plays a part.

Certain fine particles have been separated from liver cells, which absorb oxygen and give off carbon dioxide to the extent of one-fifth of that of the intact cells.

The consumption of energy by cells for the purpose of opposing the mixing of their constituents by diffusion, maintaining intact the properties, electrical or otherwise, of the semipermeable membranes and so on, must be remembered in the interpretation of the oxygen consumption by tissues which perform no external work.

There is some evidence that, in the cases of yeast and bacteria, energy is used up for the purpose of growth.

The reducing power of tissues depends to a large extent on their degree of activity, or, in other words, of the relative rate at which oxygen is consumed and supplied. The experiments of Ehrlich on the question are described briefly in the text.

When organisms emit light, it is by a process of chemi-luminescence, in which the wave length of the light is much shorter than that corresponding to the temperature of the source. It is an oxidative process in which the chemical energy is used directly for conversion to light energy, without passing through the stage of heat. The system concerned, although secreted by cells, is active apart from living protoplasm.

The nature of certain flower pigments is described in the text and their relation to oxidising and reducing enzymes indicated.

LITERATURE

Autoxidation.
Engler and Weissberg (1904).

Oxidation in the Cell.
Ehrlich (1885). Bach (1913, 1). Kastle (1910).

Reduction Processes.
Bach (1911, 2).

Influence of "Structure."
Warburg (1913, 1, and 1914).

Production of Light.
Mangold (1910). Newton Harvey (1915-1920).

CHAPTER XXI

RESPIRATION

We have seen in the previous chapters how the essential energy changes in cells are of the nature of oxidation, and we have discussed the nature of the mechanism by which ordinary molecular oxygen, arriving at the cell, is rendered active in order to burn up substances, not otherwise easily oxidised.

Oxygen must, therefore, be supplied to the cells and, in warm-blooded animals, at a considerable rate. In unicellular organisms, no special mechanism is necessary, but in larger organisms, it is clearly of importance that oxygen should be conveyed directly to the active cells, without having to diffuse through thick layers of cells, themselves consuming oxygen. At the same time, provision must be made for the escape of the carbon dioxide formed in combustion. The mechanisms concerned in this process are known as those of *respiration.*

In the majority of organisms, oxygen is conveyed to, and carbon dioxide removed from, the tissues in a state of solution of some kind in a liquid circulating through a system of tubes. This liquid is the blood. In insects, there is a system of ramifying tubes containing air. These are known as tracheæ, and the air contained in them is periodically changed by muscular movements as well as by diffusion. In organisms provided with circulating blood, there is usually a means by which free gaseous interchange of blood with the external medium containing oxygen, be it water or air, is enabled to take place. In water animals we have gills, in land animals, lungs. Arrangements are also present by which the water or air, with which the interchange of gases takes place, is periodically replaced by a fresh supply. This is done by the aid of muscular movements. The external surface of the organism not being of a sufficiently large area for gaseous exchange, special organs are developed for the purpose of affording a larger surface. The mechanisms here referred to are usually known as those of *external respiration*, as contrasted with the oxidation process in the tissues, called *internal respiration.* Intervening between the two, we have to consider the process by which oxygen is carried in the blood. The question of internal respiration is closely connected with that discussed in the preceding chapter, although there are some aspects of it more appropriately described here.

THE HISTORY OF THE DISCOVERY OF OXYGEN

That a continued supply of air is necessary to life, at all events in the higher animals, was shown clearly by Robert Hook (or Hooke) (1667, p. 539) in experiments made before the Royal Society at some of their early meetings. He showed at one meeting a dog, which was kept alive, after removal of the ribs and the diaphragm, by blowing air into the windpipe with bellows. The absence of convulsions was noted. These made their appearance when the supply of air was stopped, but were put an end to by renewing the blowing in of air. He showed also that the actual mechanical movement of the lungs had nothing to do with the recovery, as had been supposed, since he caused a continuous current of air to be blown through, and allowed to escape by means of holes pricked in the lungs. Hook himself points out that "it was not the subsiding or movelessness of the lungs that was the immediate cause of death, or the stopping of the circulation of the blood through the lungs, but the *want* of a sufficient *supply of fresh air.*" (The italics are in the original.)

The actual constituent of the air that is required was not recognised until the experiments of John Mayow (1674), who showed that it is what we now call "oxygen," but which he called "*spiritus nitro-aereus*," identifying it with that constituent on which ordinary combustion depends. Mayow was also aware that this substance entered into the blood, and was indispensable for vital activity (see Donnan's reprint, pp. 27 and 38).

FIG. 184. PORTRAIT OF JOHN MAYOW.

(Prefixed to his book, **1674.**)

The reader will probably be interested to have before him the actual words used in one or

Joseph Priestley

FIG. 185. PORTRAIT OF JOSEPH PRIESTLEY.

(From the painting by Fuseli in 1783. Copied from the print in the Yates Album in the possession of the Royal Society. By permission of the Council. The signature is from the Charter Book of the Royal Society.)

two passages. I have added the translation given in the "Alembic Club" reprint. On p. 96 of Mayow's book we read: "Nempe inprimis pro concesso habeo, Aerem particulas quasdam, quas alibi Nitro-aereas nuncupavimus, ad Ignem conflandum omnino necessarias continere; atque eas per flammæ deflagrationem ab aere exhauriri, et absumi; ita ut idem particulis istis deprivatus, in futurum ad ignem sustinendum prorsus inidoneus evadat, uti supra ostendum est." "In the first place, then, I take it for granted that the air contains certain particles termed by us elsewhere nitro-aerial which are absolutely indispensable for the production of fire, and that these in the burning of flame are drawn from the air and removed, so that the latter when deprived of these particles ceases to be fit for supporting fire, as has been shown above" (p. 67 of "Alembic Club" translation).

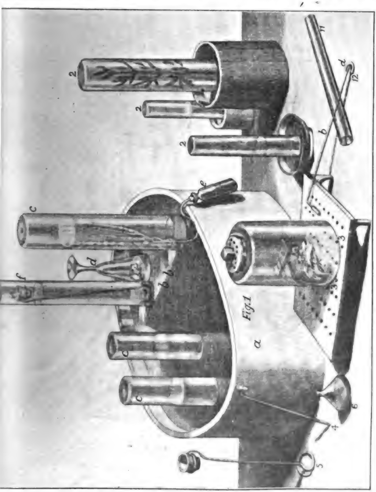


FIG. 186. FRONTISPIECE TO PRIESTLEY'S BOOK (1774).

Note the mice in the jar in the foreground and under the conical glass in the pneumatic trough, also the green plant in the jar on the right. The receptacle for reagents to insert into gases over water is seen on the extreme left.

On p. 299: "Circa respirationis ergo usum affirmare fas sit, nonnihil, quicquid sit, Aereum ad vitam sustinendam necessarium, in sanguinis massam transire. Hinc aer è pulmonibus egestus, è quo particulæ istæ vitales exhauriuntur, non amplius ad respirationem

Lavoisier

FIG. 187. LAVOISIER AND HIS WIFE.

(From the picture by David. Grimaux' "Lavoisier.")

idoneus est." "With respect, then, to the use of respiration, it may be affirmed that an aerial something, whatever it may be, essential to life, passes into the mass of the blood. And thus air driven out of the lungs, these vital particles having been drained from it, is no longer fit for breathing again" (p. 204).

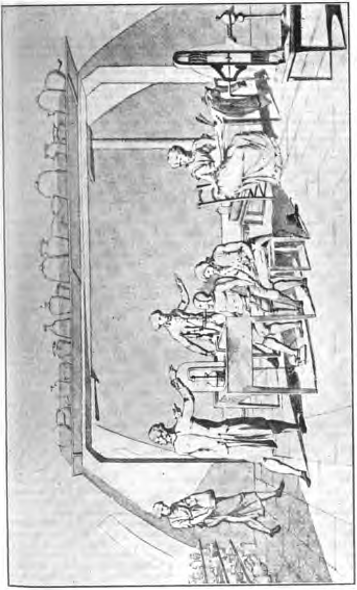


FIG. 188. LAVOISIER IN HIS LABORATORY PERFORMING AN EXPERIMENT ON THE RESPIRATORY EXCHANGE IN MUSCULAR WORK, MADAME LAVOISIER TAKING NOTES.

(From a sketch by Madame Lavoisier reproduced in Grimaux' "Lavoisier.")

On p. 107: "Ex quibus manifestum est, aerem per animalium respirationem, haud multo secus, ac per flammæ deflagrationem, vi suâ elasticâ deprivari; et utique credendum est, Animalia, Ignemque particulas e jusdem generis ex aere exhaurire; id quod sequenti experimento majus adhuc confirmatur." "Hence it is manifest that air is deprived of its elastic

force" (that is, diminished in volume) "by the breathing of animals very much in the same way as by the burning of flame. And indeed we must believe that animals and fire draw particles of the same kind from the air, as is further confirmed by the following experiment" (p. 75).

On p. 151: "Quemadmodum sanguinis fermentationem, ita etiam illius incalescentiam a particulis nitro aereis cum particulis cruoris salino-sulphureis exæstuantibus, oriri existimo." "Just as the fermentation of the blood, so also its heat arises I think from the effervescence of nitro-aerial particles with salino-sulphureous particles of the blood" (p. 104).

On p. 152: "Quanquam calor iste in animalibus, per exercitia violenta excitatus, etiam ab effervescentiâ particularum nitro-aerearum et salino-sulphurearum in partibus motricibus ortâ, partim provenit, ut alibi ostendetur." "Nevertheless, the heat excited in animals by violent exercise is in part also due to the effervescence, originating in the motor parts themselves, between the nitro-aerial particles and the salino-sulphureous particles, as will be pointed out elsewhere."

With regard to the two last passages, we must remember that, as is evident from other parts of the book, "salino-sulphureous particles" are what we now call combustible substances. It appears from the last passage that Mayow rightly held that combustion went on in the muscles themselves, although he was incorrect in his statement that it took place in the blood also. I have ventured to put this passage into slightly different words from those used by the translator of the "Alembic Club." It is clear that "effervescentiâ" agrees with "ortâ," that is, it is the "effervescence" (combustion) that arises in the muscle, not merely the salino-sulphureous particles, which would escape into the blood and be burnt there. This is a point of some importance, since it was held even by Lavoisier that the combustions take place in the lungs, so that Mayow was in advance of his successors. A portrait of Mayow is given in Fig. 184, being that placed at the front of his book.

The importance of Mayow's discovery was lost sight of in the rapid development of the phlogiston doctrine and oxygen was rediscovered by Priestley (1774), who called it "dephlogisticated air." As we have seen, Priestley also showed that air, which had been "spoilt" by animal respiration, was restored by green plants. Priestley's portrait is given in Fig. 185 and a copy of the frontispiece to his book in Fig. 186. As is well known, the doctrine of phlogiston was overthrown by Lavoisier (1770, etc.), who showed the true nature of oxidation and gave the name "oxygen" to Priestley's "dephlogisticated air." With Lavoisier, modern chemistry with its use of the balance commences. It has been stated that his discovery of oxygen was suggested by the account given to him by Priestley. However this may be, there is no doubt that he was the first, after Mayow, who saw the phenomena in their real aspect. A portrait of Lavoisier with his wife will be found in Fig. 187. The product of combustion in living beings was not known to Mayow. It was shown by Black (1755) to be something quite different from common air, and was called by him, "fixed air," but its true nature as an oxide of carbon was discovered by Lavoisier. A sketch by Madame Lavoisier of an experiment on respiratory exchange in work, performed in Lavoisier's laboratory, is reproduced in Fig. 188. Madame Lavoisier is taking notes.

As we commenced the study of the subject with the oxidation processes in the cell, it will be most appropriate to take in order the stages backwards from the cell to the lungs and the outer atmosphere.

THE STORAGE OF OXYGEN

We saw in the preceding chapter how the processes of oxidation and reduction in the cell are under the control of enzymes and the part played by peroxides therein. Thus, at a given moment, there will be a certain amount of available oxygen present in the cell as peroxide. But this must be extremely small.

At one time, it was generally thought that the cell contained a store of "intramolecular" oxygen in some loosely combined form. In our first chapter we discussed the more modern form of this belief, in the guise of "biogen" molecules, supposed to contain loosely combined oxygen in one side chain, together with combustible substance in another, so that cell oxidations might proceed without immediate supply of fresh oxygen. Since this view is still held in some quarters, occasionally in a more or less modified form, it is important to give further evidence bearing upon it.

Of course, a very small amount of oxygen may exist in the cell fluids in ordinary solution, and the time taken to consume this would vary with the rate of

oxidation in the cell. Further, a somewhat larger amount of carbon dioxide is dissolved or combined as bicarbonate. In our description of Fletcher's experiments (page 443), we have seen that the carbon dioxide given off by muscle in nitrogen is not greater than can be accounted for in the way mentioned. In absence of oxygen, therefore, no combustion process goes on in muscle; in other words, there is no oxygen present in a form available for oxidation. Further experiments on the disengagement of carbon dioxide from muscle by heat, the results of which are incapable of explanation on the theory of intra-molecular oxygen, will be found in the paper by Fletcher and Brown (1914). Similar conclusions were drawn by Verzár (1912, 3, p. 47) with regard to the muscle of warm-blooded animals. He found that there is no oxygen tension in this tissue. If there is no oxygen tension, there can be no oxygen that it is possible to use for oxidation purposes in the

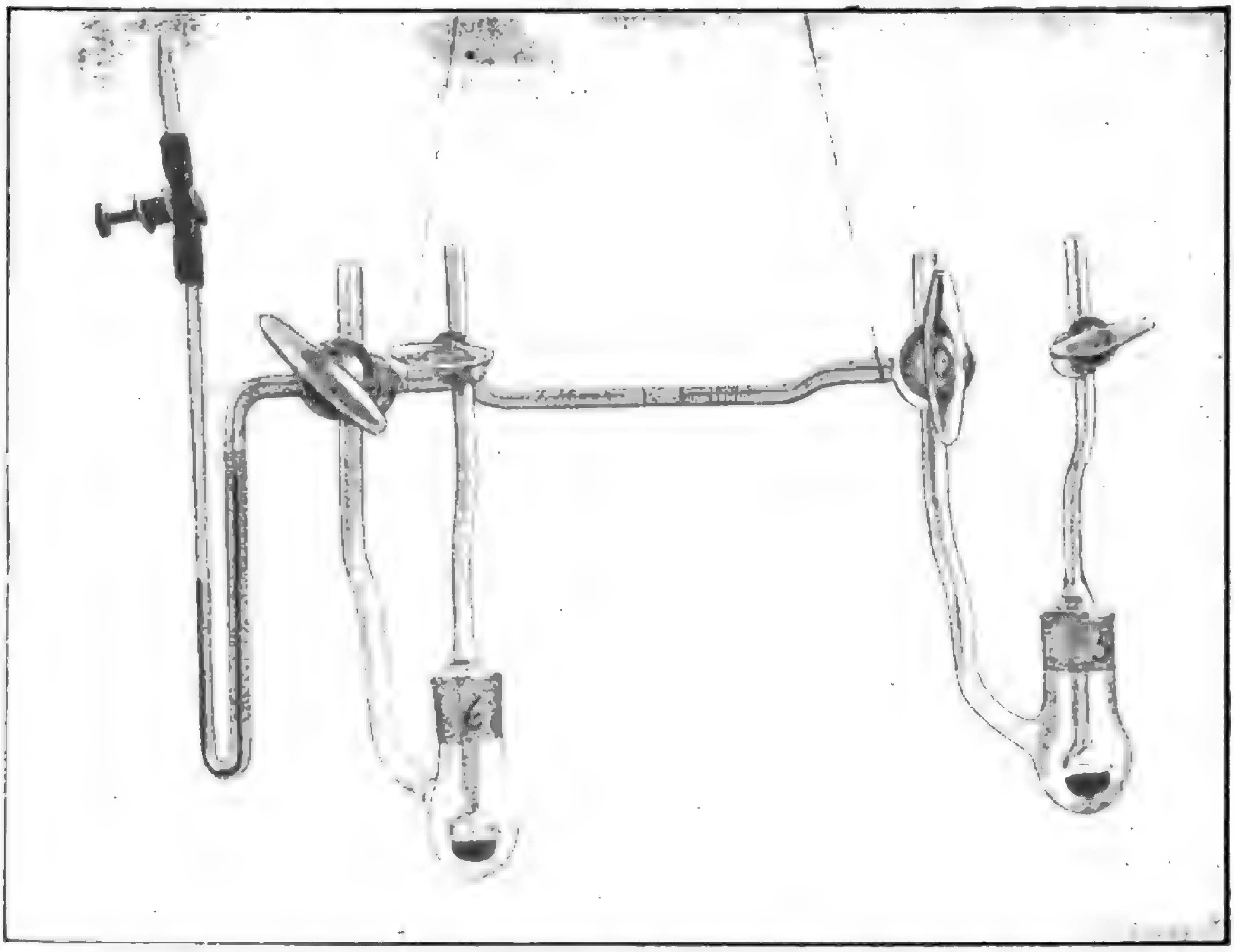


FIG. 189. WINTERSTEIN'S MICRO-RESPIRATION APPARATUS.—With tubes for introduction of various gases. To be used also for analysis of gases in small quantities of blood.

cell. There is none that can be dissociated, either for combustion of another part of the "giant" molecule or for that of other molecules. If there is any store, it is in stable combination and does not concern us here. Peters (1913, p. 266) states that his experiments do not absolutely decide the question, but he could obtain no evidence in favour of a storage of oxygen.

Turning to other tissues, the experiments of Winterstein (1907) on the spinal cord of the frog may be referred to. The micro-respirometer of Thunberg (1905, 1) was used. This apparatus is similar to that represented in Fig. 189, which is the form given to the instrument by Winterstein in order to use it for the estimation of the gases in blood, as well as for respiratory experiments. Into this apparatus the isolated spinal cord of the frog, prepared by Baglioni's method (1904), was introduced. In oxygen it remains excitable for forty-eight hours, in nitrogen only for half an hour. The oxygen consumption at 16° to 18° is 268 to 300 c.mm. per gram. The respiratory quotient is less than unity, hence some substance other

than carbohydrate is oxidised. Now, supposing that, while in oxygen, the preparation has accumulated to itself a store of oxygen in disposable form, as "biogens" or otherwise, then, when nitrogen takes the place of oxygen, this store of oxygen will be used up. Therefore, the first thing that will happen, when the nitrogen is again replaced by oxygen, will be that the store is replenished and oxygen will disappear without the corresponding amount of carbon dioxide being given off; in other words, the respiratory quotient will not be the same as that after being some time in oxygen. In very carefully controlled experiments no indication was found of any change of this kind.

It appears, then, that, although inexcitable, the tissue remains alive in nitrogen, since its excitability can be restored in oxygen. Since the survival is not an oxidative process, why is oxygen necessary for restoration of excitability? Winterstein compares it to a clock which has stopped, not because the spring has run down, but because the movement of the pendulum is hindered. In our case the hindrance is the accumulation of asphyxial products, which require oxygen to remove them. The length of time necessary for recovery is not due to slowness of diffusion of oxygen, but to the rate of oxidation of these products. Whatever they may be, it seems clear from the non-alteration of the respiratory quotient that their chemical nature is similar to that of those oxidised under normal conditions. It is scarcely necessary to remark that, after asphyxia, the rate of consumption of oxygen was temporarily increased, but the point is that the respiratory quotient was unaltered, as it would have been if oxygen were being stored apart from simultaneous production of carbon dioxide.

In the researches of Battelli and Stern (1907), however they may be interpreted, there is no evidence of storage of oxygen.

Meyerhof (1912, 1, p. 176), again, finds that in the absence of oxygen, there is no production of heat in the blood corpuscles of the goose, although it returns on admission of oxygen.

Thunberg (1905, 2), in some experiments to be referred to again later, found that the oxygen consumption of the slug and the earthworm was increased by increase of oxygen pressure, but that the carbon dioxide production was always parallel to it, so that no storage of oxygen took place, even under increased pressure.

The experiments of Falloise (1901) and of Durig (1903) are regarded by Zuntz as affording definite proof of the absence of any kind of storage of oxygen on the part of the cell.

Falloise showed that, if an animal were caused to breathe for a considerable time a mixture rich in oxygen, and then the supply cut off, symptoms of asphyxia appeared only forty-five seconds later than they did if ordinary air had been breathed. If the oxygen inhalation only lasted for one minute, the same effect resulted, and, if air were breathed for one minute *after* the oxygen inhalation, its effect was removed. Hence the only effect produced is that of the residual air in the lungs and the extra oxygen dissolved in the plasma.

Durig's experiments were made in Zuntz' laboratory by the most accurate methods and they resulted in confirming the work of Falloise. Dogs were given mixtures of air with various percentages of oxygen, and the intake per minute was determined. During the first two to three minutes after changing the mixture, the oxygen intake was increased or decreased in proportion to the oxygen content of the air breathed. Special experiments were then made to determine the amount present in the air of the lungs and that dissolved in the tissues and blood. It was found that the *whole* of that taken in or given out beyond the normal amount was required for these purposes, so that none at all was left over for storage in any other form.

We saw above (page 343) that the results of the experiments of Barcroft and Brodie (1905, p. 65) are opposed to the view of intra-molecular oxygen in this case.

Those of Evans and Ogawa (1914) are also of interest in this connection, as well as with regard to the mechanism of tissue respiration. They found that

in the heart, under the action of adrenaline, great increase both in oxygen consumption and in carbon dioxide output occurred, but that the two processes do not coincide in time. The oxygen intake reaches its maximum during the first few minutes after the drug is given, while the output of carbon dioxide reaches its maximum some time later, after the oxygen intake has begun to diminish again. The respiratory quotient is thus first lowered and then raised before returning to normal, but the mean value is unaltered. The explanation suggested is that a definite time is required for the chemical reactions which occur in the intermediate stages of oxidation, so that, if there is an increase in the rate of oxidation generally, the amount of oxygen consumed alters at once, while that of the carbon dioxide output attains its new level more slowly. This view is confirmed by the fact that, if adrenaline is continually added, the mean respiratory quotient during the administration becomes constant, but at a lower level than before the adrenaline was given. This can be seen in detail in the consideration given on p. 456 of the paper. It is merely necessary to remember that the carbon dioxide given out in a particular period does not correspond to the oxygen used in that period, but to that of an earlier period.

Certain interesting experiments on the growth of yeast by Horace Brown (1914) appear, at first sight, to show that there is, in this case, a storage of oxygen. If yeast be placed into a culture solution, which has been saturated with oxygen at its tension in air by shaking with air, the oxygen is removed rapidly and serves for subsequent combustion purposes by the yeast cells which have taken it up. In interpreting this result, it should be remembered that the amount of oxygen present in the solution was only 0·559 c.c. per cent., and also that (p. 212) it was found impossible to increase the "oxygen charge" of normal yeast, which had been washed in contact with air, by submitting it to more extensive aeration. The possibility of peroxides may be taken into account here (see the footnote on p. 212 of the paper), and also that of adsorption of oxygen on surfaces in the cell, since the amount taken up was so small.

The Relation of Oxygen Tension to its Consumption.—We find almost invariably that the supply of oxygen is sufficient to meet the requirements of the cell, so that increase of its pressure (= concentration) does not lead, by mass action, to increased consumption. In the case of the slug, the earthworm, and the mealworm, Thunberg (1905, 2) found, on the contrary, that the consumption of oxygen was, within fairly wide limits, in proportion to its tension.

NARCOSIS

Some phenomena and theories of narcosis have been discussed previously (pages 138-140). That of Verworn (1912), according to which the process consists in the inhibition of oxidation, was left until the present chapter. Allied to this view is that of Mansfeld (1909), which attributes the process to an effect on the cell membrane by which access of oxygen is prevented. This blockage is supposed to be due to the diminution of the *solubility* of oxygen in the lipoid membrane, owing to the presence of the narcotic there; but, if ordinary solution be meant, it is difficult to reconcile the view with the ordinary laws of solubility.

Direct evidence exists, moreover, which shows that there is no connection between narcosis and oxidation. Thus Warburg (1910, 2) found that, although the segmentation of the sea urchin's egg was stopped by phenyl-urethane, the consumption of oxygen was not; a greater concentration of the narcotic, however, stopped the latter also, as would be expected.

Winterstein (1913) shows that there is no relation between the narcotic action of various substances, and their effect on oxidation. Further, anaerobic worms can be anæsthetised. In a further paper (1914), it is shown that the spinal cord of the frog, when narcotised by urethane, shows diminished oxidation, but when narcotised by alcohol, the oxidation is increased. The two processes are independent. The fact that nerve centres, after asphyxia, cannot be recovered by oxygen, if alcohol be present, shows that there is some intermediate process between oxidation and excitability, which process is attacked by the narcotic.

As to what the process actually consists in, certain facts have been given previously, and we may remember that Claude Bernard (1875, p. 143) suggested that the various forms of

anæsthesia, as by drugs, heat, asphyxia, and so on, are essentially the same physico-chemical process. He also clearly pointed out that the ordinary phenomena of asphyxia have nothing to do with those of narcosis (p. 96).

Loewe (1913), as the result of detailed investigations of the relation of narcotics to lipoids, came to the conclusion that the cell membrane consists of a complex colloidal system of hydrophile colloids together with lipoids, and that the narcotics are adsorbed by the latter, with the result that their hydrophile nature is changed into a hydrophobe nature, or one that behaves as such, although no water is lost. Hence the decrease of permeability found experimentally as the accompaniment of typical narcosis, as opposed to the increase associated with lethal action. There may also be a diminution of "elective" permeability, resulting in diminution of potential difference and injury to "specific" functions of the membrane. But it is difficult to attach very definite meaning to the last statements.

ANAEROBIC EXISTENCE

We have seen that certain organisms, both animal and vegetable, such as some bacteria and nucleated red blood corpuscles, are not killed by deprivation of oxygen, although no oxidation proceeds and cell activities are suspended. Recovery takes place on admission of oxygen. In other cases, such as intestinal worms, the leech, and yeast cells, chemical activities of a special kind proceed, together with certain manifestations of life, in absence of oxygen. A further condition is that of certain bacteria, which are killed by oxygen and are capable of existence only in its absence. Thus we have facultative and obligatory anaerobiosis.

The manifestations of life require the supply of free energy. This is usually obtained from oxidative reactions, and the interesting problem arises, How is it obtained in absence of oxygen?

Perhaps the best example to start with is that of the mould, *Mucor racemosus*, which, as shown by Pasteur (1876, pp. 130-132), in the presence of oxygen burns up glucose to carbon dioxide and water, but when submerged and deprived of oxygen, certain morphological changes occur and it now forms alcohol and carbon dioxide from glucose.

Yeast.—We have seen that no growth takes place in absence of oxygen, but that the fermentation proceeds. In this fermentation, in which sugar is split into alcohol and carbon dioxide, there is production of heat; so that we may put it in this way, the combination of part of the carbon with oxygen to form carbon dioxide sets free more energy than is required to make up the difference between the heats of combustion of alcohol and of sugar. There is, then, energy at the disposal of the organism for what activities it is capable of, if this energy can be made use of. The reaction from glucose to alcohol probably passes through several stages, similar to those given on page 273.

Putrefactive Organisms.—Pasteur (1861) showed that certain organisms, responsible for butyric acid formation in putrefaction, were actually killed by oxygen, although, presumably, their spores are able to withstand its presence. In a protein undergoing putrefaction, it was shown by Hoppe-Seyler (1887) that the chemical products of putrefaction are different when the process proceeds with or without air. In the presence of air, aerobic organisms develop at the surface, while anaerobic ones grow in the depths. In presence of oxygen, carbon dioxide, water, and ammonia are formed; in its absence, hydrogen, marsh gas, leucine, and tyrosine. Nencki (1904, 1, p. 376) showed that certain aromatic derivatives, phenyl-propionic acid, parahydroxyphenylpropionic acid, and skatol-acetic acid, together with lower fatty acids, butyric, caproic, etc., were formed in anaerobic putrefaction. It is chiefly to these lower fatty acids, together with indol and skatol, that putrefactions owe their objectionable smell. Methyl-mercaptan is also sometimes present. Decarboxylation of amino-acids occurs, giving rise to various amines, and, from diamino-acids, putrescine and cadaverine (tetra- and penta-methylene-diamines).

Higher Fungi.—Kostytschev (1910) showed that mushrooms in absence of oxygen do not form alcohol. In their press juice an interesting phenomenon was observed. Carbon dioxide is formed and can be driven off by boiling. It arises only in small part from carbonates and chiefly from some substance which splits off carbon dioxide by hydrolysis. The carbamino-acids of Siegfried were excluded by the observation that the phenomenon could be observed in the absence of proteins or amino-acids. The substance in question seems to be some intermediate stage of oxidation, formed by previous exposure to oxygen. It was found that mannite disappears, if added to the press juice, without giving off carbon dioxide until the solution is heated, and it is thought probable that this substance is the source of the interesting compound in question.

Higher Plants.—Considerable evidence exists that, in absence of oxygen, higher plants attack sugar as yeast does, forming alcohol and carbon dioxide. Further details may be found in the essay by Lesser (1909).

In Animals.—Spallanzani was the first to show that animals (snails) give out carbon dioxide in an atmosphere of hydrogen or nitrogen (Foster, 1901, p. 253). We have seen (p. 444) that *muscle* in absence of oxygen undergoes no chemical change unless stimulated, and that then lactic acid only is produced. In oxygen, this lactic acid is oxidised with evolution of carbon dioxide. So that lactic acid is the product of anaerobic change, carbon dioxide that of aerobic change. But, of course, muscle cannot continue to live without oxygen.

Intestinal Worms.—These are the only multicellular animals known which normally exist in absence of oxygen. Although they have no need to produce heat, they require energy for other purposes, muscular movement, growth, and so on. They are, therefore, very instructive for investigation. The most recent work is that of Weinland (1901-1906). These worms were found to contain large quantities of glycogen, which was consumed in starvation, giving as products, in absence of oxygen, carbon dioxide as the only gas. In the liquid around the animals, valerianic acid was found, in amount corresponding to 0·3 g. per 100 g. of Ascaris in twenty-four hours, together with a nitrogenous substance containing 0·015 g. nitrogen for the same time and weight of animals. The carbon dioxide was 0·4 g. The process is represented as follows:—

$$4C_6H_{12}O_6 = 9CO_2 + 3C_5H_{10}O_2 + 9H_2.$$

The hydrogen is supposed to be used up at once for reduction processes. If this be so, we have a true fermentation process.

The Leech.—Pütter (1907) has investigated the metabolism of the leech, which can live ten days without oxygen. He states that hydrogen is formed in these conditions. When first placed in water deprived of oxygen, the carbon dioxide production goes up considerably for a time, but afterwards falls again.

Energetics of Anaerobiosis.—It appears from the preceding paragraphs that a larger amount of carbon dioxide has to be given off by a fermentation process than by an oxidation in order to give the amount of energy required by an organism. Indeed, Warburg (1914, p. 262) calculates that the same quantity of glucose when decomposed to alcohol and carbon dioxide only gives 3 to 5 per cent. of the energy which it gives when completely burnt to carbon dioxide and water. But the general conclusion seems to be justified that the cell mechanisms are such as to be able to use chemical energy whether it comes from oxidation or otherwise, and that they are independent of the particular chemical reaction which affords it.

Blackman holds (see Kidd, 1916, p. 149) that, in the respiration of plants, there are always two types, proceeding simultaneously, an oxidation of carbohydrate or fat to carbon dioxide and water (floating respiration), and a "protoplasmic" respiration, which is the necessary minimum of life. Further, there are two stages, of which the first is an anaerobic splitting of carbohydrate into carbon dioxide and an easily oxidised substance, the second is the oxidation of this substance by the oxygen of the atmosphere. Kidd shows that the narcotic action of carbon dioxide is exerted only on the first process. Thus the anaerobic products should be, as a rule, intermediate stages also passed through in the presence of oxygen, but, in its absence, undergoing no further change. This was the view suggested by Pfeffer (1881-1885, p. 664). But it does not seem to be always the case. Yeast does not ferment

any more sugar to alcohol in the absence of oxygen than in its presence (Buchner and Rapp, 1899). It may be held, nevertheless, that yeast is an abnormal organism, produced under the process of repeated selection for a special purpose. In the case of the animal cell, we have already (page 276) seen reason to hold that alcohol is not a normal stage of sugar metabolism. Further, the valerianic acid produced by Ascaris must be a special form of anaerobic metabolism. It is difficult to make any statement as to the characteristic putrefaction products, since the organisms are inactive in the presence of oxygen and we do not know what their metabolism might be in such circumstances.

THE OXYGEN CONSUMPTION OF TISSUES

We have already referred, incidentally, to the requirements of certain tissues as regards oxygen supply, both in rest and in activity. For further data, the essay by Barcroft (1908), together with his book (1914), may be consulted.

The following numbers may be of interest:—

Submaxillary Gland.—In the experiments of Barcroft and Piper (1912), the oxygen used in the resting gland amounted to 0·027 c.c. per gram per minute. The results as regards activity have been referred to above (page 342). The consumption went up to 0·089 c.c. in a particular case and continued to be raised for a hundred seconds or more after the flow of saliva has ceased. For the production of 0·3 c.c. of saliva, 0·18 c.c. of oxygen was used over and above that of the resting condition.

The Kidney.—The most recent measurement is that of Neuman (1912). Under ordinary conditions, the oxygen consumption was found to be from 0·026 to 0·06 c.c. per gram per minute. Results under stimulation to secretory activity have been given above (page 358). The increase was about four to five times that in rest.

The Liver.—Barcroft and Shore (1912) found that, in cats unfed for thirty-six hours, the oxygen consumption amounted to from 0·005 to 0·018 c.c. per gram per minute. In animals fed eighteen hours previously, 0·024 to 0·05 c.c. For the viscera drained by the portal vein, chiefly intestine, the values were 0·008 to 0·013 c.c. for the unfed, and 0·011 to 0·018 c.c. for fed animals. These facts indicate that the chief metabolism during late digestion is in the liver.

The Suprarenal Gland.—A striking fact about this organ is the rich supply of blood. Neuman (1912) found that a blood pressure of 130 mm. of mercury drives through it 6 to 7 c.c. of blood per gram per minute. This is higher than that of any other organ. Its oxygen consumption is 0·045 c.c. per gram per minute, and is increased threefold during a rise of blood pressure produced by adrenaline.

The Heart.—The chief work on this organ has been done by Rohde (1910) and by Rohde and Nagasaki (1913) on the mammalian heart perfused with Ringer's solution and by Lovatt Evans (1912, 1, and 1914, 1) on the heart-lung preparation perfused with blood. The results will be considered in a later chapter, when dealing with the mechanism of the cardiac contraction. It may be stated here that the oxygen consumed in any one contraction varies directly with the maximal tension developed, in accordance with the results of A. V. Hill (page 443) on energy production in skeletal muscle. The oxygen used per minute depends directly on the number of beats; so that, as Rohde expresses it, $\frac{Q}{NT}$ is a constant for normally beating hearts, where Q is the quantity of oxygen consumed per minute, N is the pulse rate, and T the maximum tension. The amount of oxygen consumed per gram weight, according to the results of Evans, is from 0·043 to 0·085 c.c. per minute.

The Lungs.—In the course of the above work, Evans (1912, 1) determined the metabolism of the lung tissue. This is of some importance with regard to certain theories which supposed that a considerable degree of oxidation of metabolic products of tissues took place here. It amounts only to 0·015 c.c. per gram per minute, really a low figure.

The Nerve Centres.—The metabolism of the nerve centres has been referred to

previously (page 472). Further work is required on the question, especially in connection with the great sensibility of the higher centres to deprivation of oxygen, although it has been stated that the actual consumption of oxygen is not great.

The Blood Itself.—We have already seen that the nucleated blood corpuscles have a fairly considerable oxidation metabolism. Morawitz (1909) showed that the blood of rabbits made anæmic by the injection of phenyl-hydrazine has also a fairly considerable metabolism, and that this is due to the young non-nucleated red cells which are present in such conditions in considerable numbers. In contrast with this, the actual metabolism in the normal blood is extraordinarily small.

Technique.—For the methods used in the various experiments referred to in the preceding paragraphs, the original papers must be consulted. There is a possible criticism to be brought against these methods, in which the rate of the blood flow is measured by the time taken to fill a certain volume of a graduated pipette inserted into the vein. This value is obviously of great importance in the determination of the oxygen consumed in a given time. When vascular dilatation occurs, as is usual in an active organ, the time taken to fill the tube is very short, and is only a small part of the total duration of an observation, so that the assumption must be made, that the rate of flow and consumption of oxygen continues to be the same as that during the small sample of the total effect of a stimulation which is actually measured. For this reason, it seems desirable that further observations should be made, in which the *whole* blood passing through an organ in a considerable time should be collected, and its oxygen and carbon dioxide contents compared with that of the arterial blood entering. This criticism is not intended to cast doubt on the results given above, but it seems to me that it may be quite easy to overestimate the oxygen consumption when vaso-dilatation occurs, since the measurement only applies to so short a period. The application of this consideration to the question of the nature of vaso-dilatation will be clear later, when we have to discuss the regulation of the blood supply in Chapter XXIII.

It is evident that the amount of oxygen required by active organs is far larger than the blood could carry merely in the ordinary state of solution in liquids. We have, therefore, to consider in the next place the extraordinary substance, hæmoglobin, contained in the red blood corpuscles, by whose agency oxygen in adequate amount is conveyed to the tissues. As far as difficulty of understanding is concerned, this mechanism, as we shall see, is similar to that of its near relative, chlorophyll.

HÆMOGLOBIN

Although it is this substance which is contained in the red blood corpuscles of the vertebrates, and is responsible for the taking up of oxygen, and the giving it off again when required, it is not to be supposed that there are no other similar substances. In fact, in the blood of molluscs and crustacea there is a pigment, hæmocyanin, which serves the same purpose. This pigment contains copper, whereas, as we shall see, hæmoglobin contains iron. As yet we know comparatively little about hæmocyanin, especially with regard to its relation to oxygen. It is a matter which would well repay investigation to determine whether it has the remarkable properties which hæmoglobin has in this respect, properties which are at present unique. The work of Alsberg and Clark (1914), to be given presently, indicates that hæmocyanin has not the peculiar properties of hæmoglobin.

Let us proceed to examine these properties.

Hæmoglobin, as is well known, is a compound of a protein with a complex acid substance, containing iron and pyrrol derivatives, as we have seen (page 560). We will leave, for the present, further remarks as to its chemical constitution, merely stating what is necessary for the immediate question. This is, that it exists in two forms, oxyhæmoglobin, which is regarded as a compound of the other form, hæmoglobin, or "reduced hæmoglobin," with oxygen. This oxygen can be removed by exposure to a vacuum, so that it is stated to be "loosely combined," and hæmoglobin remains.

Now, suppose that we expose blood, or a solution of hæmoglobin, to oxygen at its pressure in the air and shake together until no more oxygen is taken up. We find that, even when exposed to oxygen at a higher pressure, no more is taken up. At least, this is what is usually held to be the case, but there are very few experimental determinations which show this fact directly. At all events, it is practically "saturated," as shown by the form of the curve which we shall learn to call the "dissociation-curve."

Barcroft has recently made some determinations of the amount of oxygen taken up by blood exposed to a gaseous mixture of 85 per cent. oxygen and 15 per cent. nitrogen, and finds the following percentage degrees of saturation as compared with that regarded as complete: 102, 99, 98, and 97 in four experiments. These values were corrected for the gas physically dissolved, and point to a true saturation point. They were kindly communicated to me by the experimenter.

Next, let us take hæmoglobin, which has been saturated with oxygen at the pressure in which it exists in the atmosphere, and compare the amount of its *content in iron* with the oxygen contained. It has been satisfactorily proved by Peters (1912), in very careful and accurate work, that the amount of oxygen taken up corresponds to that required to convert the iron into FeO_2. Of course, this does not mean that the oxygen is actually combined in this way, as sometimes appears to be thought. Such a peroxide does not seem to be known and the iron is united also in organic combination. A trivalent iron might be united to two atoms of oxygen in peroxide form and the third valency attached to the organic group, but such a combination does not agree with the formula given by Küster (1912, p. 469). Too much stress must not be laid on this point, since it is difficult to see what is the function of the iron, except to combine with oxygen. It is to be remembered that the iron in hæmoglobin is not in such a form as to be electrolytically dissociated, and that it gives none of the reactions of iron salts. All that we are really justified in saying is that, when saturated with oxygen, each molecule of hæmoglobin contains two atoms of oxygen to each atom of iron, or, in other words, that each molecule of hæmoglobin takes up the same definite amount of oxygen. The work of Laidlaw (1904), however, tends to show that the iron is in different combination in reduced hæmoglobin to that in which it is in oxyhæmoglobin, since the iron-free derivative, hæmatoporphyrin, is easily obtained by the action of acid on the former, while, under the same conditions, hæmatin is obtained from the latter; that is, the iron is not split off.

But, while there is no doubt that the ratio given holds for hæmoglobin saturated with oxygen at its pressure in the atmosphere, say 160 mm. of mercury, it is a curious fact that in the presence of salts, as in the curve on p. 45 of Barcroft's book (1914), the course of the curve has the appearance of going beyond the ordinate marked 100 per cent. saturation. Is it possible that the saturation point is assumed to be that of the asymptote of the rectangular hyperbola deduced by the application of the law of mass action? As we shall see presently, this is one of the points that remains to be proved. It is quite possible that it will be found to be the case that complete saturation is attained at 160 mm. oxygen tension, but if it should be found that, under higher tensions in the presence of salts, more oxygen can be taken up than that corresponding to one molecule of oxygen to one atom of iron, the fact that this obtains at 160 mm. tension must be due to chance, certainly an unlikely possibility. Thus we have met with the first puzzle, but a more difficult one will be found immediately.

There are one or two interesting problems with regard to the function of iron in hæmoglobin which have not, so far as I know, been investigated. Hæmatin, which is hæmoglobin minus its protein constituent, but containing iron in the same form of combination, loses oxygen by the action of reducing agents and becomes hæmochromogen. This latter takes up oxygen from the air again. Now, has hæmochromogen the property which hæmoglobin has, as we shall see presently, of taking up different amounts of oxygen from different pressures? It would appear that it has not, since the oxygen of hæmatin cannot be removed by the air-pump. In fact it behaves as a chemical compound should, according to the phase rule, as we shall see. Methæmoglobin, again, contains iron in organic combination, but does not give up its oxygen to a vacuum. It has been stated that a protein, obtained from yolk of egg by Bunge, contains iron. Is it capable of taking up oxygen? Foster (1879, p. 316) refers to crystals of oxyhæmoglobin losing their oxygen in a vacuum.

Douglas, Haldane, and Haldane (1912) point out that the relative affinity of hæmoglobin for oxygen and carbon monoxide varies in different individuals. They regard this as due to the globin, since the hæmatin part is always the same. If so, it is difficult to see how the iron, which is a constituent of the latter, is alone concerned with the taking up of these gases.

Fischer and Brieger (1912) have made an interesting investigation of the behaviour of certain iron compounds to oxygen. They regard the combination of oxygen in the blood as an analogous case and that it is in the form of a peroxide, which is stable in alkaline solution, unstable in acid solution, similar to the ferrates and ferrites which they have prepared. At present, however, it is difficult to bring these results into comparison with the system of hæmoglobin and oxygen, since they were obtained by the use of hydrogen peroxide as source of oxygen, and I cannot find evidence in their work that the relative proportion of ferrate and ferrite is determined by the tension of oxygen gas.

Let us now consider the fact which has already been incidentally referred to. Let us expose hæmoglobin to oxygen at a pressure of only 10 mm. of mercury. We find that the amount of oxygen taken up by it is 55 per cent. of that present in saturation (Barcroft, 1914, p. 16). If exposed to a pressure of 40 mm. of mercury, it is 84 per cent. saturated and so on. We thus obtain a curve, such as is given in the plate opposite p. 16 of Barcroft's book (1914). This relationship was carefully worked out by Barcroft and Camis (1909), and is known as the "dissociation curve" of oxyhæmoglobin. We shall find presently that the form of the curve varies with temperature and with the presence of electrolytes, but, for the present, we will merely take the fact that the amount of oxygen taken up is in proportion to the pressure of oxygen, that is to the concentration of oxygen present in the solution.

Now this fact has not sufficiently aroused the astonishment of investigators. Assuming that oxyhæmoglobin is a chemical compound of oxygen and hæmoglobin, we naturally look around for similar ones, but, so far as chemical compounds are concerned, our search is in vain. There is none like it known to the chemist. Certain systems have, indeed, been hastily given as analogous; let us examine them, since they are instructive in themselves.

Dissociation of Calcium Carbonate.—Calcium oxide combines with carbon dioxide at ordinary temperatures to form the carbonate and, if this is heated, as in the lime kiln, the carbon dioxide is again driven off and the oxide obtained. It has been stated, probably from a misunderstanding of the table of Le Chatelier (1883), a part of which is given below, that, *at a given temperature*, different pressures of carbon dioxide are in equilibrium with different relative proportions of the carbonate and oxide, just as there are of hæmoglobin and oxyhæmoglobin in equilibrium with oxygen at different pressures, if we assume that oxyhæmoglobin is a chemical compound.

Table of Le Chatelier

Temperature.	Pressure in cm. Mercury.
547°	2·7
625°	5·6
745°	28·9
812°	76·3
865°	133·3

It is somewhat difficult to explain the meaning of the numbers in the above table without using expressions derived from the phase rule, which would tend to confuse the issue as regards our present problem. In the first place, we must confine ourselves to one temperature, as is obvious, and assume that calcium carbonate and oxyhæmoglobin are analogous; so that, taking the first line of the table, let us suppose that a temperature of 547°, with calcium carbonate, corresponds to one of 15° in the case of oxyhæmoglobin. This is, of course, admissible. Now the table states that the dissociation pressure of calcium carbonate at 547° is 2·7 cm. of mercury. That is, calcium carbonate is in equilibrium with carbon dioxide gas at that pressure, so that no change takes place. Next suppose that, without changing the temperature, we reduce the pressure of carbon dioxide to 1 cm. of mercury, and maintain it at this level by the use of a relatively large volume of gas, as we do when dealing with hæmoglobin and oxygen. What happens is that carbon dioxide comes off, and

continues to do so until the whole of the carbonate is decomposed and pure calcium oxide remains (see Findlay's book, 1904, p. 79). With oxyhæmoglobin, on the contrary, reducing the oxygen pressure does not lead to total reduction, but to a different state of equilibrium in which there is a smaller amount of oxygen "combined" with the hæmoglobin. If, again, we start with calcium oxide at 547°, and expose it to carbon dioxide at a pressure of 2·7 cm. of mercury, the *whole* is converted into carbonate; if the pressure of carbon dioxide is less than this, no change takes place at all.

If the system is a closed one, so that there is only a limited amount of carbon dioxide present, and at a pressure of 3 cm. of mercury, then a certain quantity of the gas combines with a part of the calcium oxide until the pressure is reduced to 2·7 cm. of mercury; after that, nothing further happens. But this has nothing to do with the hæmoglobin system, since oxyhæmoglobin may be in equilibrium with an unlimited atmosphere of oxygen at any pressure, and remain at the same percentage saturation indefinitely.

It is perhaps useful to state the case also in terms of mass action. A detailed account will be found on p. 55 of Cohen's book (1901) from which I take the following condensed statement. As in all heterogeneous systems, it is not a simple matter, at first sight, to understand what are to be regarded as the active masses of the constituents. That of carbon dioxide is no doubt given by its pressure. As to that of the solids, calcium carbonate and calcium oxide, the consideration of water and of naphthalene will assist. Water, in a closed space and at a given temperature, is in equilibrium with a certain definite pressure of its vapour. Naphthalene, although a solid, behaves similarly, but its vapour pressure is very small and difficult to measure. We may, therefore, assume that calcium carbonate and calcium oxide are also in equilibrium with a definite pressure of their vapours ("sublimation tension") at a particular temperature.

Now, just as the tension of water vapour is independent of the mass of the water, so are the sublimation tensions of our calcium carbonate and calcium oxide independent of their total masses. Since the chemical reaction takes place between molecules, it must be in the vapour phase surrounding the solids. At a given temperature, the concentrations of the vapours of calcium carbonate and calcium oxide are constant, being proportional to the sublimation tensions. In general, the active mass of a solid at a given temperature is therefore constant. Next, by the law of mass action, we have, in equilibrium, at a given temperature:—

$$K_1(CaCO_3) = K_2(CaO)(CO_2)$$

where the concentrations are taken as being equal to the vapour pressures. Now $(CaCO_3)$ and (CaO) are constant, hence also $K_1(CaCO_3)$ and $K_2(CaO)$ are also constant; call the former K_3 and the latter K_4 and we have:—

$$K_3 = K_4(CO_2) = K_4 \times \text{pressure of } CO_2.$$

Thus, when calcium carbonate dissociates into calcium oxide and carbon dioxide, at a given temperature, the pressure of carbon dioxide has a constant value, which is independent of the relative proportion of the two solids. This is called the *dissociation tension* of calcium carbonate at the temperature in question, and its behaviour to varied tension of carbon dioxide has been given above.

We see then that this system does not help us. It is sometimes said that it is not analogous because there are changes of phase in it; but there are also in the case of oxyhæmoglobin solutions. This substance is in the colloidal state; its particles are sufficiently large not to pass through parchment paper; it therefore possesses surface, and is a separate phase (Mines, see Barcroft, 1914, p. 51). It might be held that the molecules of hæmoglobin, being in kinetic movement, forbid the application of the phase rule. Experiments are needed as to the behaviour of hæmoglobin crystals, dry or in saturated solution. Colloids consisting of single molecules also require investigation as to their surface properties.

The Phase Rule.—As reference has been made to the application of this rule to oxyhæmoglobin, a few words are advisable to explain its general meaning. We have already seen (page 48) that, in a heterogeneous system, each component which does not mix with the others is called a *phase*, and that there is a boundary surface

of separation between the phases. What are to be regarded as the components taking part in the equilibrium is not always easy to see. They may all be of the same chemical compound, such as ice, water, and steam. In a gas phase, there may be a number of different gases, but it remains one homogeneous phase. A mixture of different solids, on the contrary, consists of as many phases as there are substances present, as in the case of calcium carbonate and calcium oxide, dealt with above. The components of the system are to be regarded as those which are not mutually dependent on one another. Thus, in the calcium carbonate case, if two of the phases are taken, the composition of the third is defined by the equation:—

$$CaCO_3 = CaO + CO_2.$$

Suppose that we have a given mass of a gas, that is, one phase, we cannot define its state by fixing one only of its independent variables, temperature, pressure, and volume. The same volume, for example, may be obtained by changing pressure and temperature inversely. But if two are fixed, then the third must have a definite value; at any given values of temperature *and* pressure, a given mass of gas can only occupy one particular volume.

Next, suppose that we have two phases, say, water in contact with its vapour. Here the condition is defined by giving one only of the variables a definite value. If we fix the temperature, the pressure under which liquid and vapour can both exist is determined also.

Finally, suppose that we have ice also, that is, three phases. We find now that it is impossible to change any one of the three variables without causing disappearance of one of the phases. In other words, there is only one temperature and one pressure at which ice, water, and steam can coexist together, the so-called "triple-point."

We see, then, that according to the number of phases present, a different number of the variable factors requires fixing in order to define perfectly the state of the system. This number is spoken of as that of the *degrees of freedom*, and a system is said to be invariant, univariant, bivariant, or multivariant according as the number of degrees of freedom is zero, one, two, or more than two.

A point of importance is that, in the heterogeneous systems dealt with by the phase rule, the state of equilibrium is *independent of the amounts* of the phases present.

Willard Gibbs formulated the *phase rule*, which may be most concisely put thus: If P is the number of the phases, F that of the degrees of freedom, and C the number of components, then,

$$P + F = C + 2,$$
$$\text{or, } F = C + 2 - P.$$

The greater the number of phases, the fewer the degrees of freedom.

In the case of water in contact with its vapour, we have two phases and one component, so that the number of degrees of freedom is,

$$F = 1 + 2 - 2 = 1.$$

In the calcium carbonate system there are two components, CaO and $CaCO_3$ (since carbon dioxide is defined by $CaCO_3 = CaO + CO_2$), but three phases, gas (there can only be one gas phase) and two solid phases. Thus,

$$F = 2 + 2 - 3 = 1.$$

Both systems are univariant, possessing one degree of freedom only. To each temperature, therefore, in both cases, there is one only definite pressure of vapour or gas with which equilibrium is possible.

In applying the phase rule to the case of hæmoglobin and oxygen, we have two solid phases, oxyhæmoglobin and hæmoglobin, if we assume that oxyhæmoglobin is a definite chemical compound. We have one gas phase, oxygen. The number of components must be two, and therefore again:

$$F = 2 + 2 - 3 = 1.$$

So that it seems that the system should behave like the calcium carbonate system,

with one degree of freedom only. But this is not in agreement with experimental results, which show that the system is bivariant; we can vary both temperature and oxygen pressure, and yet obtain equilibrium. We must either assume that, instead of three phases we have only two, or that there are three components, and it is not easy to see how this happens. Another alternative is that the phase rule does not apply to the case of micro-heterogeneous systems. There is reason to believe that curvature of surface plays a large part in the properties of the colloidal state (see page 51 above). This fact may perhaps bring reactions in which ultra-microscopic particles are concerned more into approximation to those between molecules. There may thus be a region in which transitional states between simple surface adsorption and true chemical combination are to be met with. The question requires further investigation.

The two important principles concerned, that of "mobile equilibrium" and that of Le Chatelier, have been discussed on pages 44-45.

Sodium Bicarbonate.—A solution of sodium bicarbonate in water in contact with various pressures of carbon dioxide appears at first sight to come nearer to the kind of system we want, since, even after allowing for the increased solubility of carbon dioxide with pressure, we find that more is taken up as the pressure increases and in certain proportion to the pressure, although the amount is not great and the range is a short one. A little closer examination, however, shows that the system is in no way analogous to that of hæmoglobin. In sodium bicarbonate we have a salt which is electrolytically dissociated in water, so that there is an equilibrium between the several ions and the undissociated salt. When the pressure of carbon dioxide outside is raised, more HCO_3' ions are formed in the solution. The result of this is that the dissociation is put back and more sodium exists in the state of combination as bicarbonate, as is seen by the dissociation equation:

$$(Na^{\cdot})\ (HCO_3') = K(NaHCO_3) \text{ (see page 185).}$$

In the system, therefore, there is more additional CO_2 than is to be accounted for merely by the increase of dissolved gas, but the increase is due to the fact that the salt is electrolytically dissociated. Oxyhæmoglobin does not dissociate in this way into oxygen ions, and hæmoglobin ions, and, in fact, like other proteins and amino-acids, it is an amphoteric substance, and to all intents and purposes a non-conductor. We find on p. 22 of Barcroft's book (1914) that a solution of hæmoglobin, which had only been dialysed for three days, had an electrical conductivity equal to that of 0·004 molar sodium chloride only; further dialysis would have reduced it still more.

Reducible Dyestuffs.—There are a number of dyestuffs which are capable of existing in two forms, an oxidised and a reduced form. In the presence of oxygen, in many cases, the reduced form (leuco-base) is oxidised. Prof. W. A. Osborne informs me that he hoped to find amongst these a case like hæmoglobin, but was unable. All of them behaved like calcium carbonate; that is, under a given oxygen pressure, the dye was either completely oxidised or completely reduced, according to the pressure. Again a case of all or nothing. Further work is required, especially as to whether dyes in the solid state or in colloidal solution behave differently from those in true solution.

According to the work of Alsberg and Clark (1914), hæmocyanin is similar to these dyes. It is blue in the arteries and colourless in the veins, that is, the reduced form takes up oxygen and becomes blue. But this oxygen is not given off to a vacuum; the blood merely gives up the gas dissolved in the water. It is suggested that the copper contained in the pigment may act as a catalyst, as we have seen above (page 585), the oxygen being thus more readily given off to an acceptor, such as may be present in the tissues. If so, hæmocyanin would be analogous to a peroxide-peroxidase system. The work of Bottazzi (1919) and of Dhéré (1918) show that there are complicating conditions present in blood containing hæmocyanin.

Adsorption.—It may occur to the reader that there is one class of cases of which

no mention has yet been made, namely, the taking up of gases by surfaces such as that of charcoal, adsorption, in which we certainly get a relation between the amount taken up and the pressure. This was, in fact, suggested by Wolfgang Ostwald (1908) as applying to the hæmoglobin-oxygen system. But it is obvious that it is very difficult to reconcile the fact that one molecule of hæmoglobin, when saturated, combines with one molecule of oxygen and no more, with anything but a chemical compound *as the final result.* The key to the puzzle will probably be found in a combination of the two processes. The amount of oxyhæmoglobin would be determined by the amount of oxygen adsorbed on the surface of the hæmoglobin under a given pressure. At the same time, there are difficulties in the treatment of the problem from this point of view, but it has, as yet, received little attention. It seems clear that it is not permissible to use either the law of mass action or the phase rule as applying to the case, until it has been proved that they do or do not hold in the case of colloidal solutions, where there must be surface phenomena intervening, although these phenomena may not be as simple as when larger and flatter surfaces are concerned.

Taking pure hæmoglobin in solution, and regarding the oxygen dissolved under various pressures as its concentration, which is, by Henry's law, a function of the pressures, Barcroft finds (1914, pp. 17-23) that the relative amounts of hæmoglobin and of oxyhæmoglobin which are present under a given oxygen pressure are in accordance with the law of mass action. The curve is a rectangular hyperbola. Under the hypothesis of adsorption, we should expect a parabolic curve. Under certain conditions, as we shall see presently, results are obtained which correspond more closely with such a curve. The greatest difficulty in the simple adsorption hypothesis is, however, that already mentioned, namely, the ratio of oxygen to iron or hæmoglobin in complete saturation.

However this may be, in respect to the function of hæmoglobin in the organism, the precise way in which oxygen is attached to it is of less importance than the investigation of the ease and rapidity with which oxygen is taken up from the air and passed on to the cells. It is especially here that the work of Barcroft and his coadjutors on the dissociation curve, as modified by various agencies, is of inestimable value.

Before passing on to these important practical questions, it may be pointed out that it has been shown that some colloidal solutions take up gases in greater proportion than is to be accounted for by the increase of solubility with pressure. The experiments of Findlay (1908) may be mentioned. Those of Geffcken (1904) are also to the point. It may be asked why, if the taking up of oxygen by hæmoglobin is conditioned by a surface adsorption, other colloidal constituents of the blood do not show a similar behaviour? Now, Geffcken's experiments indicate a case which appears to be a typical one of adsorption, namely, that of carbon dioxide by colloidal ferric hydroxide, but which is more or less "specific," in the sense that oxygen is not taken up by the solution in any larger amount than by pure water. This system of carbon dioxide and ferric hydroxide would repay further investigation, especially from the point of view of reversibility. Granting that it is one of adsorption, we must remember that this process is due to a diminution of surface energy *of any kind,* so that, as already pointed out, chemical combination on the surface, if associated with diminution of surface energy, would take place. But this does not really help us in the hæmoglobin problem, because we are still faced with the same difficulty of equilibrium with different oxygen tensions; the hypothetical chemical compound is merely changed in position, so to speak. Moreover, it is not easy to see how a permanent equilibrium could be established, since the compound on the surface must interchange sooner or later with the molecules inside the aggregate. Is it possible that, after all, there may be some state of combination, neither mere surface adsorption nor chemical in the true sense, but intermediate between them, as appears to have been held by van Bemmelen and by Ostwald? The effects of great curvature of the surface of colloidal particles may be called to mind. The interesting views of Langmuir (p. 64) on the nature of adsorption are of importance in the present connection.

Relation to Temperature.—Under a given oxygen pressure, it is found that less oxygen is taken up by hæmoglobin the higher the temperature. A series of curves will be found in Fig. 190, from Barcroft's book (1914, p. 36). This is clearly of importance with regard to the giving up of oxygen to the tissues. Suppose that blood at 38° has come into equilibrium with an oxygen tension of 100 mm. of mercury in the alveolar air of the lungs. It will be 93 per cent. saturated. From the experiments of Verzár (1912, 3), we find that the oxygen

tension in tissues varies from zero to some 10 to 20 mm. of mercury, dependent, of course, on the rate at which it is consumed in relation to that at which it is supplied. Take the case of 10 mm. From curve IV of the figure we see that at this tension hæmoglobin is only 56 per cent. saturated, so that the difference between 56 per cent. and 93 per cent., namely 37 per cent., represents that available for the tissue. On the contrary, take curve II, at 25°; at 100 mm., we have about 98 per cent. saturation; at 10 mm. 88 per cent., a difference of 10 per cent. only. The advantage of the warm-blooded animal is plain.

The different position of the equilibrium at different temperatures must

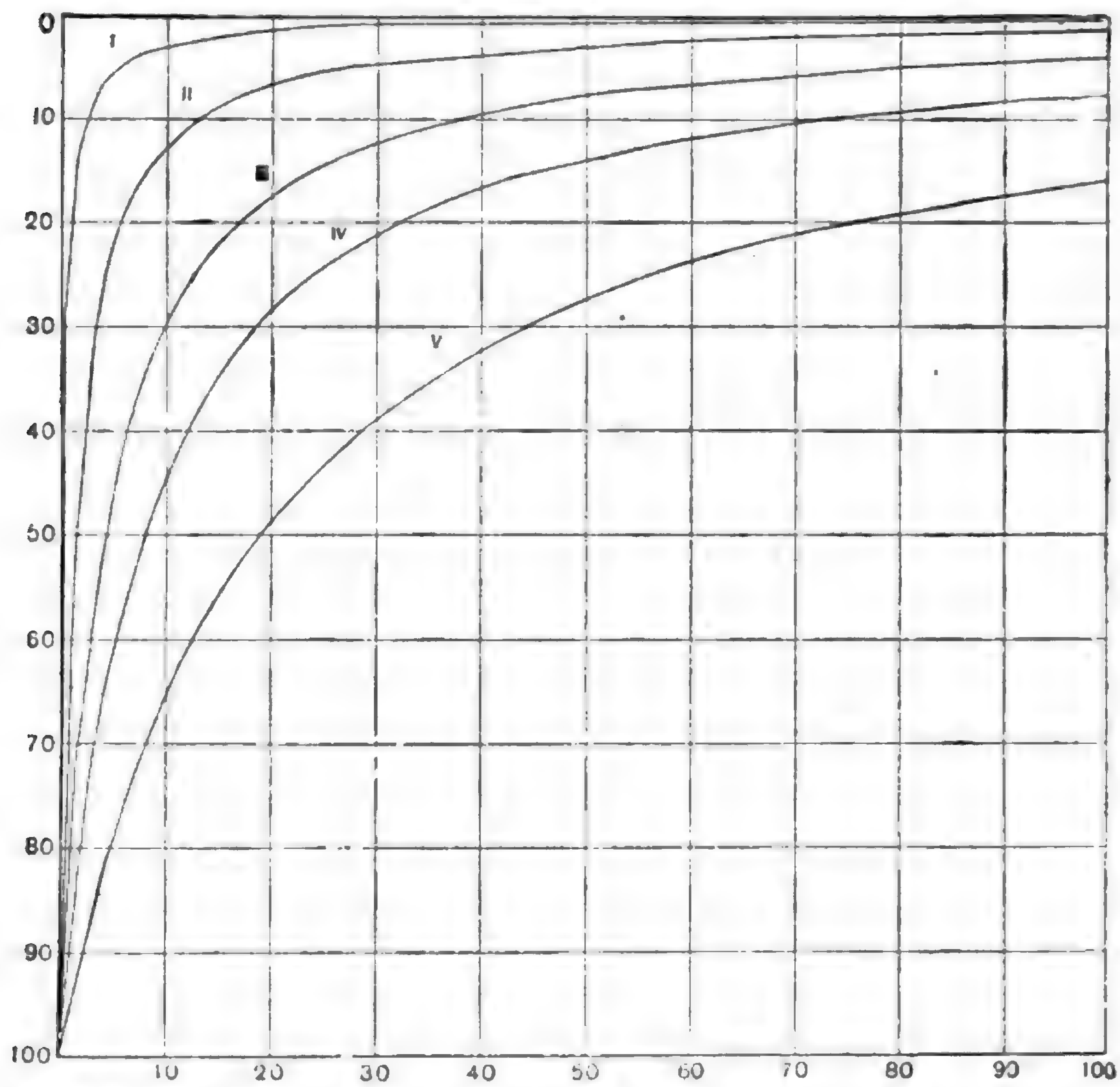


Fig. 190. Dissociation curves of oxyhæmoglobin at different temperatures.

Ordinates—percentage of reduced hæmoglobin.
Abscissæ—tension of oxygen in mm. of mercury.
Curves I, II, III, IV, and V correspond to 16°, 25°, 32°, 38°, and 49° C. respectively.

Note that the higher the temperature, the less oxygen is held by hæmoglobin at a given tension of the gas.

(Barcroft and Hill, *Jl. Physiol.*, 39, 422.)

obviously be due to the greater acceleration by temperature of the dissociation of oxyhæmoglobin than that of the taking up of oxygen. Experiments on this question will be found in Barcroft's book (1914, Chapter XI.), together with curves.

It may be noted that the effect of temperature is the same as that on cases of typical adsorption, where it is due to the negative temperature coefficient of surface energy.

Now, since raising the temperature causes dissociation of oxyhæmoglobin, van't Hoff's principle of mobile equilibrium tells us that the "combination" must be associated with evolution of heat. Further, van't Hoff has worked out a formula relating the position of equilibrium to the heat evolved on combination.

Barcroft and Hill (see Barcroft's book, 1914, Chapter III.) made experiments to determine the heat evolution, and found a value of 1·85 calories per gram of hæmoglobin. From the formula of van't Hoff, it is possible to calculate the molecular weight of hæmoglobin, on the assumption that each gram combines with 1·34 c.c. of oxygen. The result came out nearly identical with the accepted molecular weight, 16,669, and it is clear that it affords considerable support to the view of true chemical combination. But here we come across another puzzle.

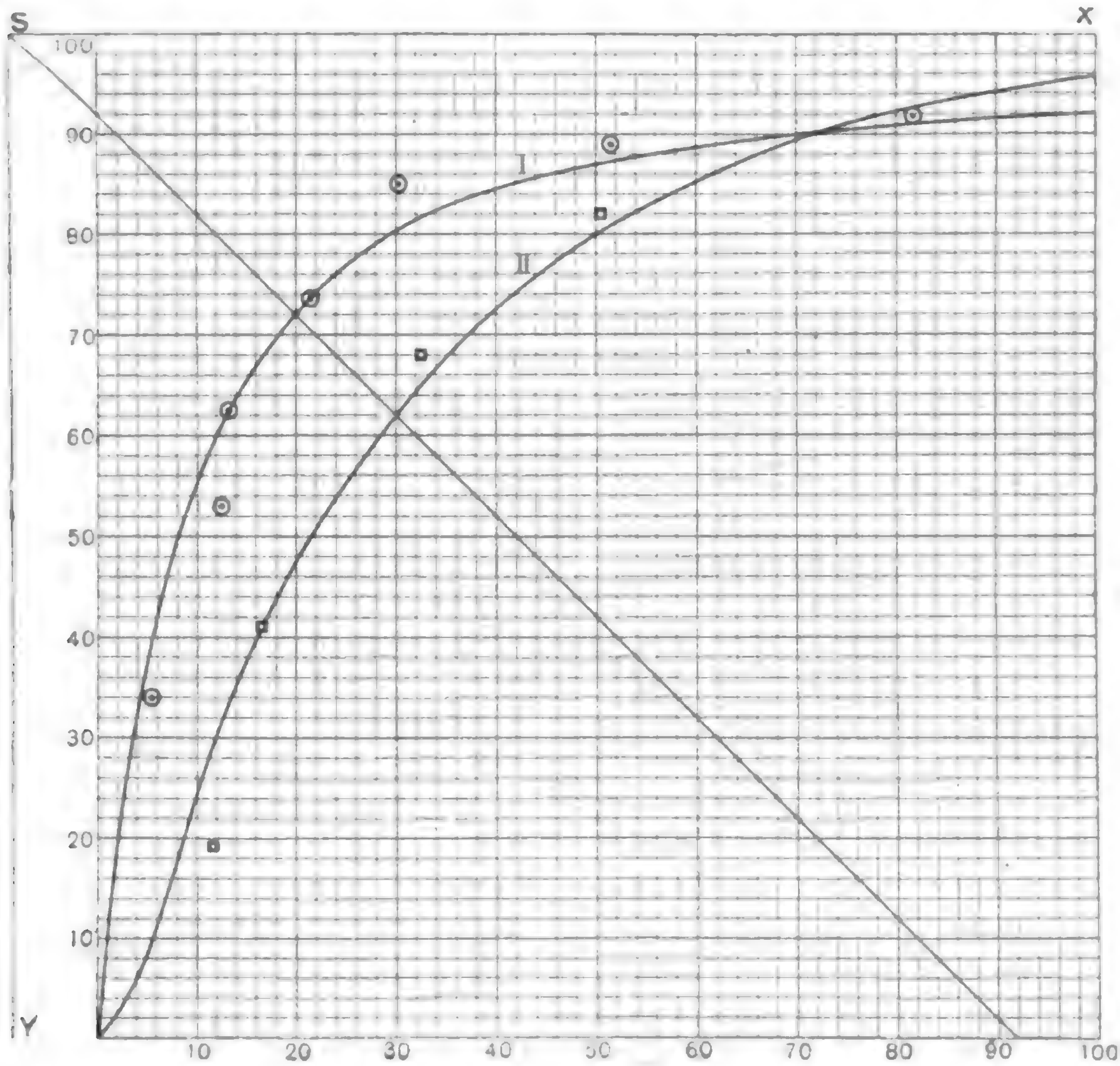


Fig. 191. Effect of electrolytes on the dissociation curve of hæmoglobin.

Ordinates—percentage saturation of hæmoglobin with oxygen.
Abscissæ—tension of oxygen in mm. mercury.
⊙—curve from dialysed solution.
□—curve from undialysed solution.

The first curve (electrolytes absent) corresponds to Hüfner's curve and is a rectangular hyperbola. It passes very nearly through the experimental values.

The second curve (salts present, in low concentration) is Bohr's curve.

The difference between the degree of saturation is especially marked at the lower oxygen tension.

(Barcroft and Roberts, *Jl. Physiol.*, 39, 146.)

The heat of combination of oxygen and hæmoglobin has been determined by other experimenters, and results considerably lower than that mentioned have been obtained; the numbers may be found in Meyerhof's paper (1912, 1, p. 164). If we consider only that of Torup (1906), which was obtained by a method essentially the same as that of Barcroft and Hill, and there is no apparent reason to doubt the accuracy of the determination, we find only 0·678 calorie per gram. R. du Bois-Reymond (1914) found values between 1·06 and 1·77, in the mean, 1·36.

In the consideration of the problem we must not forget that the condensation of gases on

surfaces (adsorption) is also accompanied by the evolution of heat, as would indeed be expected from the compression, or perhaps liquefaction, involved. If we take, for example, the values obtained by Titoff (1910), we find that the heat evolved in the adsorption of various gases by charcoal is of the same order as the values of the "heat of combination" of hæmoglobin with oxygen. Thus: at 0°, 1 g. of charcoal adsorbed 0·259 c.c. of nitrogen under a pressure of 10·2 mm. of mercury, with a development of heat of 0·373 calorie per c.c. adsorbed. The corresponding values for carbon dioxide and ammonia are about 0·33 and 0·4 calorie. One gram of hæmoglobin at room temperature takes up 1·34 c.c. of oxygen, and gives off 1·85 calories, that is, 1·37 calories per c.c. If we take Torup's result, we have 0·41 calorie per c.c. oxygen taken up. I merely call attention to the fact, without drawing conclusions.

Effect of Salts and of Acid.—The dissociation curve of pure hæmoglobin, as we have seen, can be expressed by the equation to a rectangular hyperbola. If, however, we compare this curve with that given by Bohr for hæmoglobin as present in blood, we see that the latter has a different shape. Now, it was shown by Barcroft and Roberts (see Barcroft's book, 1914, p. 22) that Bohr's curve is correct for normal blood, and that if the blood is dialysed, the first form, similar to that obtained by Hüfner with pure solutions of hæmoglobin, is obtained. Fig. 191 is a reproduction of one given by Barcroft and Roberts.

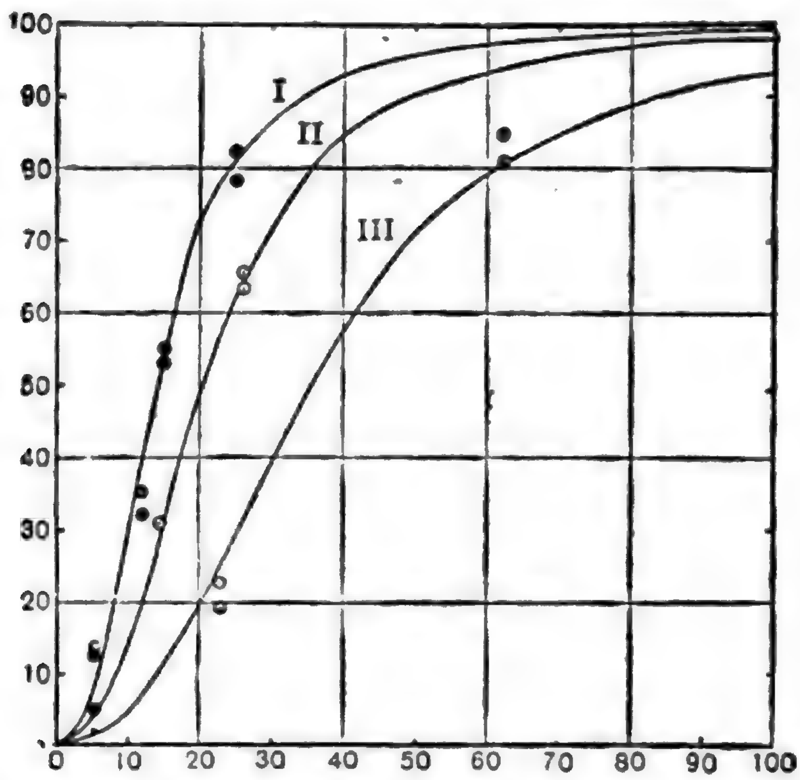


FIG. 192. EFFECT OF CARBON DIOXIDE ON THE DISSOCIATION CURVE OF HÆMOGLOBIN IN THE BLOOD.

Ordinates—percentage saturation.
Abscissæ—oxygen tension.
Uppermost curve with 3 mm. mercury carbon dioxide tension.
Middle curve with 20 mm.
Lowest curve with 90 mm.

(Barcroft and Poulton, "Proc. Physiol. Soc." in *Jl. Physiol.*, 46, p. iv.)

The physiological importance of this fact is similar to that referred to above in connection with temperature. In the presence of salts, hæmoglobin gives off its oxygen more readily, so that if the oxygen tension in the tissues has fallen to 10 mm. of mercury, the percentage saturation of the hæmoglobin of the blood may be reduced to 25 per cent., whereas, if the hæmoglobin were in pure solution in water, it would only be reduced to 55 per cent. of saturation.

The effect of acid is the same as that of salts, but more marked (see Fig. 192). Investigation shows that the effect is due to the hydrogen ions. Again, its importance is obvious. All cells produce carbon dioxide in activity and muscle in particular produces lactic acid. Both facilitate the giving off of oxygen to the active cells.

Christiansen, Douglas, and Haldane (1913) show that the amount of carbon dioxide taken up by blood is greater by one-tenth when the hæmoglobin is reduced than when saturated with oxygen. Venous blood can, therefore, take up more carbon dioxide at the same tension than arterial blood can. As the blood takes up oxygen again in the lungs, this carbon dioxide is more easily given off. From the adsorption point of view, this fact is not difficult to explain. According to Freundlich (1909, p. 116), and the experimental results of Hempel and Vater (1912), from a mixture of solutes each constituent is adsorbed and the relative proportion is governed by their relative powers of lowering surface energy, but even that one which lowers surface energy most is adsorbed *less* than from a pure solution. Hence it appears that the lower the tension of oxygen, the more carbon dioxide would be adsorbed, and the lower that of carbon dioxide, the

more oxygen. Each gas, in fact, assists to drive off the other, but we require more knowledge of the relative lowering of surface energy at the hæmoglobin-plasma boundary effected by the two gases before we can make any calculations on this basis.

The form of the dissociation curve is very sensitive to the concentration of hydrogen ions, so that it can be used as an indicator for changes in this direction occurring in the blood, either as the result of muscular work, of want of oxygen, or in pathological states of "acidosis."

Now, what are the equations to the curves obtained in the presence of acid or of salts? Since hæmoglobin is in colloidal solution and, as we have seen (page 91), electrolytes have a powerful effect in causing aggregation of colloidal particles, this phenomenon would naturally be looked for as the explanation.

A. V. Hill (1910, 2), on the hypothesis of the aggregation of molecules of hæmoglobin causing the reaction to become of a higher order than unimolecular, arrived at an expression of the form :—

$$y = 100 \frac{Kx^n}{1 + Kx^n},$$

where y is the percentage saturation of hæmoglobin with oxygen, x the oxygen pressure. This formula, by proper choice of the constants, K and n, was found to apply to the experimental data of several cases taken.

In attempting to understand the meaning of this equation, it is well to point out that Hill himself (p. vi) did not profess to attach any direct physical meaning to the constants, although Barcroft (1913, p. 481) regards K as the equilibrium constant and n as the average number of molecules of hæmoglobin in each aggregate. Hill subsequently adopts this view to a large extent (1913, 5).

It must be confessed that it is a very difficult matter to grasp the conditions under which the various states of equilibrium in a colloidal system are attained, and any criticism that I may make as to the above-given interpretation must not be misunderstood. It is, undoubtedly, an extremely valuable contribution to the theory, but careful consideration has made it clear to me that some doubtful assumptions are made, and that a satisfactory solution of the problem will only be reached by taking account of the conditions prevailing at the boundary surfaces of the phases of a heterogeneous system, micro-heterogeneous, it is true, and that the law of mass action alone is insufficient. If the phase rule requires special proof in its application to colloidal systems, so also does the simple law of mass action.

It is clear that Hill's formula applies to the curves obtained by experiment. Looking at those of Figs. 191 and 192, we see at once that, under the influence of electrolytes, the dissociation curve is no longer the rectangular hyperbola of a unimolecular reaction. But why should mere aggregation of hæmoglobin change the order of the reaction? As I understand the theory of velocity of reaction, as based on mass action, the order would be changed only if molecules of a *different chemical kind* came in to take part in the reaction. There seems no reason to suppose that the various degrees of aggregation of hæmoglobin result in change of its chemical nature. With regard to oxygen, of course, no suggestion of this kind is possible. On the other hand, it is not impossible that aggregates may from the kinetic standpoint, behave as different chemical individuals. So that too much stress must not be laid on this difficulty. If we grant that Hb_2O_4 is not the same as two molecules of HbO_2, then the treatment of the question by mass action is justifiable.

There are two forms of equation already known to us in which we have an exponent, which we have called n in both cases. The first is that expressing the velocity of reaction, where it has the significance of the number of different kinds of molecules taking part in the reaction, whose concentration may vary independently, so that it is necessary to take account of the change in concentration of each. In this case it must, naturally, be a whole number. The second equation is that expressing the amount of a substance adsorbed by a surface as a function of the concentration of the substance. In this case, n

may be, and usually is, fractional. The curve in both cases belongs to the parabolic family, but, if we glance at those of Figs. 191 and 192, we see that, in the presence of salts or acid, the experimental curve is S-shaped, that is, more complex than either of these two possibilities alone.

In the data given by A. V. Hill (1910, 2), we note at once that the values of n are mostly fractional. The explanation given is that there are present a number of aggregates of hæmoglobin containing different numbers of molecules, so that the net result is a combination of different orders of reaction above the unimolecular one. But, as already pointed out, it is not clear that it is legitimate to assume that the n of Hill's formula corresponds to that of the expression for the velocity of reaction, where it refers to the number of kinds of molecules taking part. If aggregation takes place, it would seem more likely to produce a change in the effective concentration, rather than in the exponent; that is, in C in the formula :—

$$\frac{dx}{dt}=kC^n,$$

and therefore in the equilibrium constant, K, of Hill's formula.

Put in another way, it is not obvious why the order of the reaction,

$$Hb_2+2O_2 \rightleftharpoons Hb_2O_4,$$

should differ from that of

$$Hb+O_2 \rightleftharpoons HbO_2,$$

unless Hb_2 is a different chemical individual from Hb, and that it dissociates differently. But, as already pointed out, this may be so (see Barcroft, 1914, p. 60).

In the reaction :

$$\bar{E}t\bar{A}+H_2O \rightleftharpoons \bar{E}tOH+H\bar{A},$$

although the association of water and of alcohol differs at different temperatures, there is no evidence of a change in the order of the reaction, so far as I am aware

A. V. Hill, in a further note (1914, 3), shows how the same equation,

$$y=\frac{Kx^n}{1+Kx^n},$$

can be obtained thermodynamically by consideration of osmotic pressures, without reference to aggregation. But the applicability of mass action to the system is assumed, and the difficulty lies here rather than in the hypothesis of aggregation, which is not improbable. It is further suggested that the lowering of osmotic pressure required in this form of treatment might be due to the unequal distribution of electrolytes, described above in relation to Congo-red (page 160), but the electrolytic nature of oxyhæmoglobin is not yet demonstrated. The situation of the membrane is not clear when none is provided by the experimenter, although the boundary between the gas phase and the liquid phase may act as such.

With reference to the constancy of n (about 2·5) in the presence of different concentrations of carbon dioxide (see Barcroft's book, 1914, pp. 65 and 66), it is scarcely necessary to add that, in itself, no proof is hereby given that it is explicable as the order of a reaction. As Barcroft remarks, "since n remains so constant, it is probably the expression of some definite physical fact," and it seems to me that this is as far as we can go at present.

The constancy of n with a particular acid leads Barcroft to make the statement (1913, p. 490) that the action of acid does not lead to change in the number of molecules in the aggregates, but to a change of the equilibrium constant. But, as we have seen, it is not satisfactorily shown that n refers to the number of molecules in the aggregates, and I might venture to point out that constancy of the exponent is also a characteristic of adsorption. From the similarity of the curves in the cases of the action of acids and of salts, one would infer that, whatever the action may be, it differs only in degree in the two cases. It might be thought that, as a part of the hæmoglobin molecule is of protein nature, this would enter into combination with acid; but, as we have seen (p. 103), there is no evidence that proteins or amino-acids, except the strongly basic ones, combine with weak acids at all. Perhaps measurements of the electrical conductivity of hæmoglobin solutions, as changed by the action of acids, might throw light on the question. Barcroft also suggests (1914, p. 316) that the $H^{\cdot}$ ion causes the globin molecule to aggregate, and itself enters into combination with

the hæmatin constituent. Hæmatin itself appears to have acid properties, so that it seems difficult to accept this suggestion.

On the whole, it is clear that much more work is necessary before we can regard the nature of the association between oxygen and hæmoglobin as decided. I have felt it necessary to point out where existing hypotheses fail, though it would have been pleasanter to be able to take them as satisfactory. There seems some risk that the question may be considered, prematurely, to be settled. At the same time, I have no alternative hypothesis to suggest, although I cannot help thinking that the subject would repay more investigation from the adsorption point of view than it has yet received. Not having worked at it myself, I hold no brief for one side or the other, and cannot claim any particular value for my remarks, which are merely based on the aspects presented to an onlooker.

The Action of Carbon Monoxide.—This gas has, as it is expressed, a much greater "affinity" for hæmoglobin than oxygen has, so that, in a mixture of the two gases, there is a much larger amount of carbon monoxide combined with hæmoglobin than corresponds to the relative tension of the two gases. At the same time, there is a definite law regulating the proportion, which has been made the basis of a method of determining the oxygen tension of arterial blood by Douglas and Haldane (1912). According to Nicloux (1913, 1914), the relative proportions of carboxyhæmoglobin and oxyhæmoglobin is regulated by mass action.

With regard to adsorption from mixtures (pages 70 and 622 above), it is interesting to note that carbon monoxide is very strongly adsorbed by many solid catalysts (Bancroft, 1918, 3), acting as a poison by driving off the reacting constituents from the surface.

Optical Properties.—Hæmoglobin and its derivatives give very definite absorption spectra. In Fig. 193 a series of photographs is given. The fact is of practical value in the colorimetric and spectro-photometric methods of estimation of hæmoglobin in general use. One cannot, at present, assign any significance to the absorption of light from the photo-chemical point of view, except that, as we saw above (page 571), the absorption of ultra-violet light has probably a protective function.

Hartridge and Hill (1914) have made interesting observations on the infra-red absorption of hæmoglobin, comparing it with that of reduced hæmoglobin and the compound with carbon monoxide. They find that it is considerable in this region, which has great radiation-energy, and that the absorption of carboxyhæmoglobin is only about half that of oxyhæmoglobin. It is clear that determinations of the absorption in this region of the spectrum would enable estimations to be made of the relative amounts of the three substances present in a solution, a point of practical importance, as we shall see later in connection with the oxygen tension in blood. The measurements would be made by a thermopile, as described in the catalogue of Messrs Adam Hilger. This infra-red absorption is of interest in another way. Light produces a change in the equilibrium between oxygen, carbon monoxide, and hæmoglobin, as shown by Haldane and Lorrain Smith. We have seen that, by Nernst's formula (1913, p. 679), we can calculate the free energy of a reaction, if we know the equilibrium constant. Therefore, we have here a photo-chemical reaction, in which light energy can be stored. Hartridge and Hill calculate, from the known change of the equilibrium constant, in the above reaction, what this amount of energy is, and find that it is very considerable, in fact, much greater than that of any similar photo-chemical reaction, with the exception of that of the chlorophyll system.

Chemical Constitution.—This question was discussed briefly in Chapter XIX. (pages 560-561) in relation to chlorophyll, and the meaning of the iron content was referred to in an earlier part of the present chapter (page 614).

Methods of Investigation.—A useful account of the methods used in the determination of the degree of oxygen saturation of hæmoglobin is given in detail in the appendix to Barcroft's book (1914). The apparatus of Winterstein (1912, 1), especially with the later improvements (1913, 2), is, in many respects, very convenient in use, both for blood gas analysis and for respiratory exchange of small organs. It is more fragile than that of Barcroft (see Fig. 189 above). Krogh's apparatus (1916, p. 23) is an excellent one.

THE LUNGS

We have seen how oxygen is conveyed to the tissues, by the agency of hæmoglobin, in greater quantity than could be done if it were merely dissolved

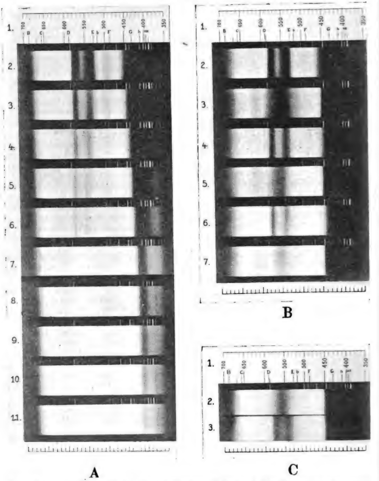


Fig. 193. Absorption spectra of hæmoglobin.—Photographed with grating spectrograph.

A—1, Wave length scale.
2-11, Series of dilutions of rabbit's blood, oxygenated. Dilutions range from 1 : 70 to 1 : 2,000. The ultra-violet band is seen in the more dilute specimens.
Thickness of layer, 14 mm.

B—1, Wave length scale.
2, Normal blood, 1 : 70.
3, The same reduced with ammonium sulphide. The delicate band in the orange is due to sulph-hæmoglobin.
4, Normal blood, 1 : 100.
5, The same reduced.
6, Normal blood, 1 : 150.
7, The same reduced.

C—1, Wave length scale.
2, Normal blood, 1 : 200 reduced.
3, Blood exposed to carbon monoxide and then acted upon by ammonium sulphide. No change in the bands.
The bright line spectra are those of a helium tube.

(After Rost, Franz, und Heise.)

in the blood-plasma, and how oxyhæmoglobin gives up oxygen to places where the tension of the gas is lower than that where the oxygen was taken up by the hæmoglobin. It remains to consider the mechanism by which hæmoglobin, after being robbed of the greater part of its oxygen by the tissues, replenishes its supply from the external air. Incidentally, the carbon dioxide which has been given off to the blood by the cells escapes to the atmosphere at the same time. As we shall see later (p. 635) carbon dioxide is transported almost entirely by the same substance, hæmoglobin, as conveys oxygen. Of course, owing to the greater solubility of carbon dioxide, a somewhat larger amount is dissolved in the water of the blood.

It is generally known that, in air-breathing animals, there are arrangements by which a large surface of blood is brought into contact with air, which is itself repeatedly changed. A thin membrane is all that intervenes, so that the distance through which the gases have to diffuse is extremely short. The organs in which this interchange takes place are known as lungs.

I quoted above the experiment of Hooke, in which he showed that a renewed supply of air is necessary to preserve an animal from death by asphyxia. It does not belong to the subject matter of this book to describe the details of the muscular mechanisms by which the air is sucked in and expelled from the lungs. Suffice it to say that their capacity is periodically increased and diminished by the action of muscles on the walls of the cavity in which they are contained.

It will be obvious that the whole of the air cannot be expelled in expiration unless the lungs are squeezed flat, a mechanical impossibility in the construction of an animal. The air in the final terminations of the branching air tubes, the alveolar air sacs, must possess, therefore, a tension in oxygen lower than that of the atmosphere, and one of carbon dioxide higher than that of the atmosphere. It is with this air that the gases in the blood enter into exchange. The problem before us is, then, how do the oxygen and carbon dioxide tensions of the arterial blood leaving the lungs compare with those of the alveolar air?

Since a gas always diffuses from a place of higher tension to one of lower tension, it is clear that if the pulmonary exchange is regulated by the laws of diffusion alone, the oxygen tension of the arterial blood can never exceed that of the alveolar air, and that its carbon dioxide tension can never fall below it.

In the first place, it is important to grasp the meaning of the *tension of a gas* in a fluid, as opposed to its actual concentration. In a mixture of gases at atmospheric pressure the matter is simple, the tension of any one is proportional directly to its relative concentration. Thus, oxygen makes up 21 per cent. of the air, and therefore its tension at the ordinary atmospheric pressure is 21 per cent. of 760 mm., that is, 159·6 mm. of mercury; and, in fact, it is 21 per cent. of any pressure under which air may be placed. Now suppose that we have a volume of gas at 760 mm. pressure containing 10 per cent. of carbon dioxide, its tension is 76 mm. Place the gas next in contact with a layer of water, and allow equilibrium to be attained, keeping the tension of the carbon dioxide in the gas phase constant by adding more as required. The water dissolves a certain quantity of the carbon dioxide, and, at its contact surface with the gas phase, a certain number of molecules of carbon dioxide are continually entering the water and a certain number leaving it, so that equilibrium means that the same number enter and leave in the same time. It follows that the tension of the carbon dioxide is the same in both phases, although if we determine its total concentration in the water and in the gas, we shall not find it to be the same. Further, let us add some alkali to the water, still keeping the carbon dioxide tension in the gas phase constant; as is well known, carbon dioxide combines with alkali to form carbonate and bicarbonate, so that the liquid phase will contain much more carbon dioxide than the gas phase per unit volume, but again the tension at the surface, and therefore throughout the liquid, must be identical with that in the gas mixture when equilibrium is reached. We may deal with oxygen in the same way, supposing hæmoglobin to be present in the liquid instead of the bicarbonate.

What we have to do is to determine the oxygen and carbon dioxide tensions in arterial blood, and compare them with the alveolar tensions of the two gases. The methods used to do this are the carbon monoxide method of Haldane and Douglas, already referred to, and the aerotonometer methods. The latter consist in exposing the blood to a limited volume of gas, of a composition as nearly as possible the same as regards the tensions of its components as that of the blood. After equilibrium has been attained, the composition of the gas phase is estimated by the usual methods of gas analysis. It is doubtful whether the earlier experiments were trustworthy, since the volume of the gas was too large. Those of Krogh (1910) are free from this objection. He devised a method by which a small bubble of gas can be analysed with the greatest accuracy. This bubble of gas was exposed to the current of arterial blood, being kept in constant motion by the current. After a time it was transferred to the measuring tube, and the carbon dioxide and oxygen contained in it determined. The result was that the tension of oxygen in arterial blood, under the conditions of the experiments, was always *lower* than that of the alveolar air. Hence, so far, there is no difficulty in the diffusion theory. The tension of carbon dioxide was found practically identical with that of the alveolar air, but never less. The actual value of the carbon dioxide tension will be referred to again later.

At this point we must consider the view taken by Bohr, who believed that his experiments showed that the alveolar epithelium has the power of actively secreting oxygen in the direction of the blood, so that the tension of oxygen in the arterial blood may be higher than in the alveolar air. Certain physiologists, Haldane and Douglas, to mention two only, still hold this view in a modified way. While admitting that the evidence is against the secretion of oxygen under ordinary conditions of rest and even of temporary want of oxygen, as in muscular exercise, they hold (see especially Douglas, Haldane, Henderson, and Schneider, 1913, pp. 204 and 205) that, during the process of acclimatisation to a high altitude, with its low oxygen tension, the lung epithelium develops the power of secreting oxygen. The table given on p. 197 of the paper named gives a number of data, and it will be seen that the oxygen pressures in arterial blood, as determined by the carbon monoxide method, are considerably higher than in the alveolar air in all the cases which had become acclimatised.

It is a difficult matter to understand how such a function should have been formed in the course of evolution to meet a need very rarely arising. We must remember that it is only supposed to show itself after exposure to want of oxygen for a considerable time. We may also consider briefly some further objections brought by Krogh against the view. In the first place, Krogh points out that, owing to the form of the dissociation curve of oxyhæmoglobin, the hæmoglobin is nearly saturated at the ordinary alveolar tension of oxygen, so that, in order to increase the oxygen percentage by 0·4 per cent. only, an increase of tension of 30 per cent. would be necessary. Of course, this is not so serious an objection when the alveolar oxygen tension is as low as that on the top of Pike's Peak, namely, about 60 mm.; but, even at this pressure, the hæmoglobin is 86 per cent. saturated. In any case, it seems a poor result for a new and special mechanism to be formed. As far as concerns the actual work required, A. V. Hill (1913, 3) shows that what is actually necessary to raise the oxygen tension from that of the alveoli to that of the arterial blood in Douglas, Haldane, Henderson, and Schneider's experiments is a very small fraction of that done by the organism as a whole. The value is given by the expression we have frequently made use of:—

$$W = RT \log_e \frac{p_2}{p_1},$$

where p_2 is the higher pressure and, of course, the integral has to be taken along the particular limits of the dissociation curve corresponding to the respective tensions. In man, the amount of energy per minute works out at about one gram calorie. This might be done by epithelial cells of 0·5 μ in thickness, if their efficiency were 20 per cent., that is, no greater than that of the body as a whole. Another criticism made by Krogh is that the histological structure of the pulmonary

epithelium is not at all what would be expected in a secreting organ. The cells are quite thin, and very unlike those of the gas gland of the fish, where, as we have seen (page 361), there is an obvious reason why oxygen should be actively secreted. A gas must be produced and absorbed in adaptation to the pressure at different depths. It has been said indeed that, in the bird, where the need of extra supply of oxygen would be supposed to be greater than in the mammal, the pulmonary alveoli are devoid of epithelial lining altogether. If the exchange were by diffusion alone, the direct contact of the walls of the blood capillaries with the alveolar air would be of advantage. Again, unlike a secreting gland, oxygen passes with equal facility in either direction. Breathing fire damp, for example, causes instantaneous unconsciousness through loss of oxygen from the blood to the gas in the alveoli.

Hartridge (1912, 1) introduced an improvement in the carbon monoxide method of Douglas and Haldane, by substituting observation of the change of position of the absorption bands, which is produced by carbon monoxide, instead of the mere visual comparison of the colour of two solutions. Using his new method, Hartridge (1912, 2) investigated the effects of producing oxygen want in the tissues in three ways, by breathing mixtures containing carbon monoxide, by lowering the oxygen tension of the air breathed, and by doing work. He was unable to find any evidence of oxygen secretion by the lungs in any case, but, as was stated above, Douglas and Haldane now hold that it is not to be detected until acclimatisation has been developed.

Bohr introduced the consideration of the rate at which oxygen could pass through the pulmonary epithelium and capillary wall, and calculated, entirely from theoretical data, what he called "invasion" and "evasion" coefficients. The conclusion to which he came was that the difference between the tension of oxygen in the arterial blood and that in the alveolar air could only be accounted for by secretion on the part of the cells. Krogh, however (1910, 1), made direct experiments on the rate at which oxygen passed from water into a gas bubble, and found that the "invasion coefficient" is really nearly seven times that calculated by Bohr. It seems possible that the solubility of oxygen in water, which enters into the formula, is altered at the contact surface between the epithelium and the alveolar air, owing to the action of surface forces, a fact neglected by Bohr. We saw above that the solubility of gases depends on the surface tension of the liquid solvent (page 54), and that a low surface tension increases the solubility (Christov). It is quite possible that the surface tension of the liquid covering the membrane of the lung alveoli may have a very low surface tension, owing to presence of lipoid. If this were so, the solubility of oxygen in it might be much greater than that reckoned by Bohr. From the invasion coefficient it can be calculated how much oxygen can pass into the blood in a given time, and, although it appears that it is sufficient to satisfy the conditions of rest on the diffusion theory, it is held by Barcroft (1914, p. 216) that diffusion will not account for the large amount of oxygen used in exercise, or under the conditions of low oxygen tension as in rarefied air. It is to be remembered that the calculation requires knowledge of the quantity of blood passing through the lungs. Krogh and Lindhard (1912) determined this experimentally in man, and found that, in muscular work, it might rise to as much as 21·6 litres per minute, instead of the much smaller number taken by Bohr (1909) as the basis of his calculation. On p. 228 we find the following calculation. In a particular experiment it was found that 162 c.c. of oxygen per litre of blood passing through the lungs was taken up and utilised; that is, 85 per cent. of the difference between arterial and venous blood. In muscular work, 2,700 c.c. of oxygen were consumed per minute. Hence, if we take 21 litres per minute as the cardiac output, according to the measurements of Krogh, we find that $162 \times 21 = 3{,}400$ c.c. of oxygen per minute can be taken up by the lungs—more than enough to satisfy requirements. Similarly, the work of Patterson and Starling (1914) shows that the amount of blood sent out by the heart, when working under optimal conditions, is very much larger than previously assumed. Taking the data available, it can be shown that the amount of oxygen which the blood can carry from the alveolar air by diffusion is considerably in excess of that found to be consumed under any muscular work hitherto determined. Marie Krogh (1915) has made further experiments and finds that diffusion is quite capable of explaining the maximum amount of oxygen consumed in muscular work (see also Bainbridge, 1919, pp. 160-169).

At the same time, it must be admitted that we have no explanation for the results of Douglas and Haldane on Pike's Peak. It seems very desirable that the experiments should be repeated on lower animals by the aerotonometer method of Krogh. The difficulty is that the animals must be kept for some time under reduced oxygen pressure and the experiments made under anæsthesia. As regards the latter factor, the adherents of the secretion theory may make the objection that the narcosis paralyses the secretory power of the cells; but it has no such effect on other glands. There is one fact in the data given by

Douglas, Haldane, Henderson, and Schneider which seems a little strange, although it may have no significance. Notwithstanding that the arterial oxygen tension was always higher than that given for the alveolar air, it was never as high as that of the atmosphere at the time, although occasionally not much below it. Why should the secretory power fail just at this level and not raise the oxygen tension above that of the atmosphere? Is it possible that the blood had come into equilibrium with oxygen tension somewhere which was not given correctly by the measurement of that of the alveolar air?

Might it not also be possible that the carbon monoxide method gives different values when the hæmoglobin content of the blood is increased, as in the case of acclimatisation to high altitudes? Hasselbalch (1912) shows that the hydrogen ion concentration is increased under these circumstances. However this may be, the question seems to be decided by the experiments of Barcroft, Cooke, etc. (1920). Barcroft lived for six days in a chamber in which the oxygen tension was reduced to 84 mm. Samples of the arterial blood at the end of the time, both in rest and in muscular work, showed a *lower* oxygen tension than that of the alveolar air. Thus, the direct aerotonometer comparison has now been made, and on man himself.

This question of secretion by the lungs is instructive from the point of view of "vitalism." When first proposed, it was held to apply to the ordinary state of affairs; but, as improvements were made in experimental methods, the absorption was shown to follow physical laws: it was then held to apply to cases of muscular exercise, and now only to acclimatisation to high altitudes. The more accurate the methods of investigation, the better is it found that chemical and physical laws are capable of explaining physiological phenomena.

THE REGULATION OF RESPIRATION

By Hydrogen Ion Concentration of the Blood.—The renewal of the air with which the blood interchanges its gaseous constituents is effected by muscular movements, and it is plain that the rate of change of the air in the lungs needs to be varied in order to provide for the different rates at which oxygen is consumed and carbon dioxide evolved in states of rest and of activity. How is this done?

The co-ordination of the muscular movements required is effected by the "respiratory centre" in the bulb, which sends out periodic discharges to the motor neurones of the spinal segments in which the muscles concerned are represented. Like other nerve centres, this centre is capable of being influenced by afferent impulses, especially from the lungs themselves (see page 632 below).

It is the great merit of Haldane and Priestley (1905) to have shown that the regulation of respiration, meaning by that the amount of ventilation per unit of time, or the total volume of air sent in and out of the lungs, is effected by the carbon dioxide tension of the arterial blood, which is the same as that of the alveolar air of the lungs. Later work showed that the hydrogen ion concentration, due to the dissolved carbon dioxide, is the actual exciting agent. The cells or synapses of the respiratory centre must, therefore, be very sensitive to hydrogen ions.

Now, the venous blood from the organs does not pass directly to the centre, but only after having interchanged with the alveolar air. The carbonic acid tension of the arterial blood is then the determining factor of the ventilation. It is thus of some importance to know how this value is related to that of the alveolar air, and this again to that of the venous blood. Bohr thought it necessary to assume, along with oxygen secretion, an active excretion of carbon dioxide on the part of the pulmonary epithelium. Krogh's experiments, already mentioned, showed the carbon dioxide tension of the arterial blood to be equal to that of the alveolar air, not *less*, as it would be if actively excreted. He points out that the remarkable sensibility of the respiratory centre to a slight increase of the carbon dioxide tension of the alveolar air would be upset by interference with the relation between that of the alveolar air and that of the arterial blood, such as would result from an excretory process. Haldane and Priestley, in fact, showed that a rise of the carbon dioxide tension in the lung alveoli of only 1·6 mm. of mercury, or of 0·22 per cent. of its content in carbon dioxide, increases the ventilation of the lungs to double its previous value. If the carbon dioxide tension of the venous blood rises by a very small amount, that of the alveolar air will also rise by diffusion, so that the arterial blood leaving the lungs will have a

slightly higher tension in carbon dioxide. At once the respiratory centre is stimulated, and more copious ventilation rapidly washes away the excess of carbon dioxide from the alveoli, and thus from the venous blood.

That it is to changes in the hydrogen ion concentration that the respiratory centre reacts is, perhaps, most definitely shown by the experiments of Hasselbalch (1912), although previous workers had found that the centre responds to acids other than carbon dioxide. Reference to these results will be found in the paper by Hasselbalch. Those of Winterstein (1911, p. 179) may be mentioned. He found that respiratory movements could be induced in rabbits, four days old, which were perfused with oxygenated Ringer's solution from the aorta, when 0·001 molar hydrochloric acid was added to the solution, although no carbon dioxide was present. The nature of one kind of proof brought by Hasselbalch will be clear from the following consideration. Since a particular carbon dioxide tension in the alveoli corresponds to a definite ventilation, when other things are unaltered, it follows that, if we find this same ventilation along with a lower carbon dioxide alveolar tension, some other cause must be adding its influence on the centre. Hasselbalch found that, by altering the diet, he could alter the hydrogen ion concentration of the urine, hence that of the blood, which he also measured by the hydrogen electrode described above (page 192). The carbon dioxide of the alveolar air always varied inversely with this hydrogen ion concentration; hence the lung ventilation is always adjusted in such a way as to maintain the hydrogen ion concentration of the blood constant. Since, normally, the alveolar carbon dioxide tension varies only in very narrow limits, the sensibility of the kidney to acid in the blood must be such as to keep the concentration of hydrogen ion in the blood, other than that due to carbon dioxide, at a constant level.

Certain observers (see Bainbridge, 1919, p. 28) give evidence that carbon dioxide is a more efficient stimulus than corresponds to its H ion concentration. The suggestion made by Jacobs (1920) has been referred to on page 218.

It may, perhaps, seem surprising that it is to carbon dioxide rather than to oxygen tension that the respiratory centre is adjusted since it is oxygen that is the chief requirement. Various observers have sought for a sensibility of the centre to a fall of oxygen tension; the conclusion arrived at has been that, until the oxygen tension falls very low, and the products of tissue activity are not completely oxidised, no increase of ventilation takes place, provided that increase of carbon dioxide tension is prevented. Although the centre is not sensitive to fall in oxygen tension in the sense of being excited by it, it is possible that its excitability might be raised so that the same carbon dioxide tension which excited it normally under normal oxygen tension might, under reduced oxygen tension, excite greater activity. Campbell, Douglas, Haldane, and Hobson (1913) showed that the alveolar oxygen pressure can be varied within wide limits without sensibly affecting the excitability of the respiratory centre to carbon dioxide.

This want of response to lowered oxygen tension may, under certain conditions, lead to serious consequences, such as mountain sickness, which will be referred to later. It is the increase in carbon dioxide tension that produces the increase of pulmonary ventilation in asphyxia, so that, if increase in carbon dioxide be prevented, as by respiration of pure nitrogen, a man may become unconscious before experiencing any unpleasant symptoms. Similarly, by forced breathing, the carbon dioxide tension may be reduced to such an extent, that so long a period of absence of stimulus to the respiratory centre may ensue, that severe signs of want of oxygen show themselves. Krogh points out that any mechanism of active excretion ought to be stopped when death threatens owing to loss of carbon dioxide.

It was mentioned above that, when the oxygen supply to the tissues is considerably below their requirements, acid products are formed, especially in the muscles; these products naturally play a part in the stimulation of respiration by their hydrogen ion content. Thus Ryffel (1909) has shown that lactic acid is

to be found in the urine after vigorous muscular exercise. When the oxygen tension in the blood has fallen below about 60 mm., the nerve centres are excited and convulsions ensue. This result is due to the formation of asphyxial products, probably of an acid nature, in the centres themselves. Mathison (1910, 1911) studied the stimulation of the spinal and bulbar centres by deprivation of oxygen without increase of carbon dioxide, that is, by respiration of nitrogen, and came to the conclusion that it is acid formed in the centres themselves which excites them. The bulbar (vasomotor) centre is more easily excited than the spinal

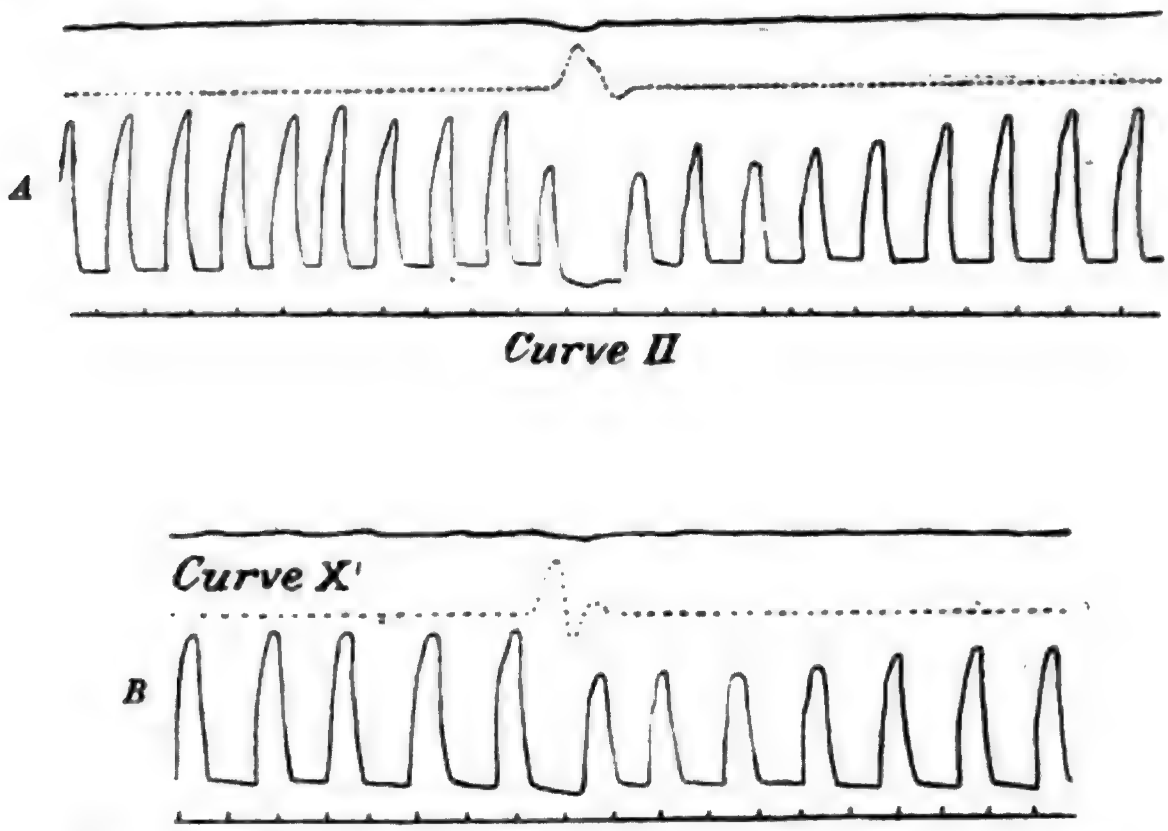


FIG. 194. RESPIRATORY REFLEXES PRODUCED BY INFLATING THE LUNGS.

A, The dotted line is the tracing of a mercury manometer connected with the trachea, a rise indicating distension of the lungs.
The curve below it is that of a lever attached to the diaphragm slips of the rabbit, and represents the movement of the diaphragm as a whole. Thus inspiration is shown by an upward movement of the curve.
The tracing at the top is from a control lever attached to the walls of the chest.
Note that distension inhibits inspiration.

B, Similar effect of short inflation with pure hydrogen. To show that the result of the preceding experiment was not due to cessation of respiration owing to increased supply of air.

(Head, 1889, Pl. 2, Figs. 2 and 10.)

centres. Thus, the former reacts to thirty seconds' deprivation of oxygen, to 5 per cent. carbon dioxide, or to 2 c.c. $\frac{n}{20}$ lactic acid. The spinal centres require two minutes' deprivation of oxygen, 30 per cent. of carbon dioxide or 5 c.c. $\frac{n}{6}$ lactic acid. The production of acids other than carbon dioxide is a sudden one and occurs at the point when the cell mechanisms are beginning to be disorganised; hence the process is not of use to the organism, as far as the centres referred to are concerned, although the acids formed are very potent stimuli.

For further details with regard to the chemical regulation of respiration, the essay by Douglas (1914) may be consulted.

The Nervous Mechanism.—From what we have seen above (pages 500, 511, and 540) as to the importance of afferent impulses from contracting muscles (proprioceptive impulses) in the regulation of their activity, it would be surprising if the respiratory mechanism were devoid of similar control.

In the present case the receptors are mainly in the lungs and in connection

with the respiratory centre by means of the vagus nerves. They have been referred to above (page 387) in the discussion of the meaning of the electrical changes in the peripheral ends of the vagus nerves, as photographed by Einthoven (see Fig. 106, p. 386). The relative parts played by the nervous and the chemical factors were the subject of investigations by F. H. Scott (1908). Although expiration, under normal conditions, is almost entirely a passive movement, due to return of the mechanism to its equilibrium position, it is well known that, in dyspnœa, co-ordinated action of muscles antagonistic to those of inspiration takes place in the expiratory phase. We must, therefore, suppose that the respiratory centre is double, inspiratory and expiratory, and each of these will be capable of excitation and of inhibition, forming a system subject to double reciprocal innervation of the kind described above (page 498) with reference to the flexors and extensors of the limbs.

Although it had been known for a long time that respiratory movements could be influenced reflexly by stimulation of various afferent nerves, and especially of the vagus, the first clear and systematic results were obtained by Head (1889), using a slip of the diaphragm, which is so attached in the rabbit as to allow a tracing lever to be connected without interference of the nervous supply to it or disturbance of the normal respiratory movements. The original paper must be consulted for numerous points to which we cannot refer here. The main question is as to what happens when the vagus endings in the lungs are stimulated by expansion and collapse of these organs. Hering and Breuer (1868) had already made experiments of this kind with a less perfect method, and founded a theory of self-regulation by the vagus nerves. Head showed definitely that the effect of *increase in the volume* of the lungs is to *inhibit* all inspiratory movements, and that *decrease of volume* has the opposite effect of *exciting* inspiration (see Figs. 194 and 195). Of course, as we should expect from what is known now of the part played by carbon dioxide, either inflation or suction, repeated periodically, produces stoppage of spontaneous respiration, owing to removal of carbon dioxide, but, with the vagi intact, the stoppage is in the position of inspiration (= excitation of inspiratory centre), with suction, and the opposite with inflation (see Head's diagram, Fig. 195A).

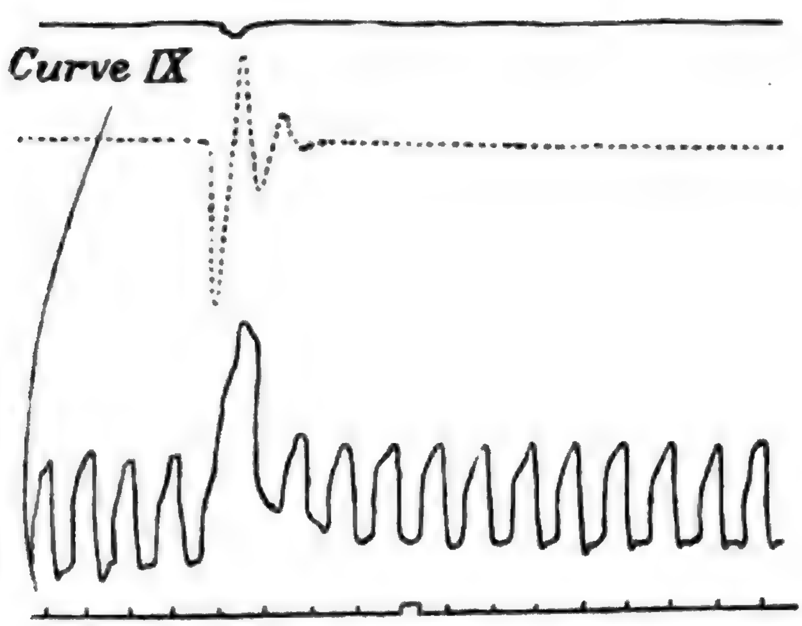


Fig. 195. Effect of short suction.—As above in regard to meaning of tracings.

Note that deflation excites inspiratory movements.

(Head, 1889, Pl. 2, Fig. 9.)

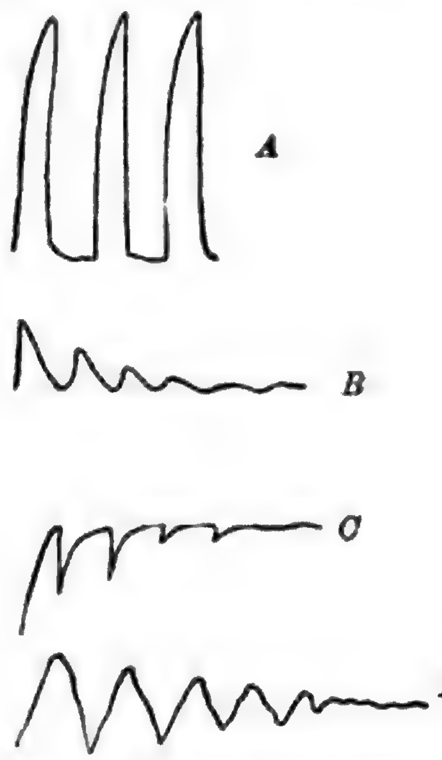


Fig. 195A. Diagram of effects of ventilation on respiratory reflexes. — Inspiration upwards.

A, Normal tracing of diaphragm slip.
B, Inflation. Standstill in relaxation.
C, Suction. Standstill in tonic contraction.
D, Combination of the two. Cessation of automatic movements. Diaphragm in mean state of tonus.

(Head, 1889, p. 31.)

Scott's experiments (1908) showed that the increased respiratory activity produced by inhalation of carbon dioxide differs in form when the vagi are cut from that when they are intact. In the former case the respirations are increased in depth, without increase in rate, whereas there is an increase in both rate and depth when the vagi are intact. It would appear, then, that the inspirations excited by carbon dioxide in the normal state are cut short by the vagi inhibiting the discharge of the centre; collapse of the lungs follows rapidly, the inhibition ceases, and the centre is again accessible to excitation by carbon dioxide. In this way we have the advantage of an increased rate in addition to the increased depth. Carbon dioxide does not cause the centre to discharge more frequently, but with increased strength of discharge. The function of the nervous regulation is thus to moderate the discharge, which tends to be "all or none," in the absence of inhibitory impulses.

In muscular exercise, increased ventilation is chiefly provided by increased depth. In Benedict and Cathcart's experiments (1913), we find that the ventilation may be increased tenfold while the rate only rises from 20 to 30 per minute.

Apnœa.—Since the stimulus to the respiratory centre depends on the tension of carbon dioxide in the alveolar air, it is plain that a diminution of this tension causes cessation of respiratory movements. This is true apnœa. Considerable discussion took place at one time as to whether a summation of the inhibitory vagus impulses described above, could produce stoppage of respiration. Campbell, Douglas, Haldane, and Hobson (1913) have shown that no apnœa can be produced, at least in man, unless the alveolar carbon dioxide tension is reduced.

EFFECTS OF WANT OF OXYGEN

Some aspects of this question have been referred to, incidentally, in previous pages. A few words may be added here with respect to "*mountain sickness.*" In a low atmospheric pressure, not only is the oxygen tension below normal, but the alveolar carbon dioxide is also naturally at diminished pressure. Consequently, the stimulus to the respiratory centre is absent or very weak, although increased supply of air is necessary. The organism suffers, therefore, from want of oxygen. The symptoms observed on Pike's Peak are described thus by Douglas, Haldane, Henderson, and Schneider (1913, p. 308): constant blueness of lips and face, loss of appetite, nausea and vomiting, intestinal disturbance, headache, sometimes fainting, periodic breathing, and great hypernœa on exertion. Difficulty in mental effort is also experienced. All of these symptoms are such as are produced by want of oxygen. The view of Mosso that "acapnia" or diminution of carbon dioxide is the directly responsible factor can no longer be held (see especially the book by Zuntz, Loewy, Müller, and Caspari, 1906). The symptoms are almost identical with those of carbon monoxide poisoning, "which have been shown to be due to want of oxygen and to nothing else." The psychical disturbances are often like those of alcoholic intoxication; unreasonable behaviour on the part of visitors required the presence of a deputy-sheriff on Pike's Peak. Perhaps unreasonable people met with under ordinary circumstances are really suffering from want of oxygen.

The first proof that the symptoms and dangers of low barometric pressure depend on diminished oxygen pressure, and consequent insufficient oxygen content in the blood, was given by Paul Bert (1878).

In acclimatisation, the acidity of the blood due to non-volatile acids is increased, so that the respiratory centre becomes properly excited, although the alveolar carbon dioxide tension is low. At the same time, the hæmoglobin content of the blood is increased, as shown by Paul Bert (1882). Dallwig, Kolls, and Loevenhart (1915) describe increase in the activity of the red bone marrow, by which the number of the red blood corpuscles is raised under lowered oxygen tension.

It will be obvious that the danger from lack of oxygen is much greater in balloon ascents, where no time for acclimatisation is given. The tragic ascent of Tissandier with two companions in 1875 will be remembered (see Paul Bert, 1878, p. 1063). Some of the words used by Tissandier are worth quoting as typical of the state of oxygen deficiency. "At about 7,500 m. (300 mm.

barometric pressure) the condition of torpor which comes over one is extraordinary. There is no suffering—one rises and is glad to be rising. This state of exhilaration continues to the last moment, and immediately precedes loss of consciousness, sudden, unexpected, and irresistible." When Tissandier recovered consciousness, both of his companions were dead. Although all were provided with oxygen to inhale, they were paralysed before they were aware of the fact, and therefore unable to take hold of the tubes.

THE TRANSPORT OF CARBON DIOXIDE

Although much attention has been directed to the transport of oxygen by hæmoglobin, it is remarkable that it has, almost universally, been assumed that it is the bicarbonates in blood that serve as the carriers of carbon dioxide. We shall see presently that this is very doubtful.

Notwithstanding the fact that Setschenov in 1879, and Bohr in 1887, showed that hæmoglobin is capable of taking up and giving off carbon dioxide (see Bohr's article in Nagel's "Handbuch," 1909), the circumstance that Bohr attributed the function to the protein component, and the apparent belief that the combination of hæmoglobin with oxygen, being a chemical one, excluded the possibility of its being able to take up carbon dioxide in a way similar to that in which it takes up oxygen, have led to the neglect of what has recently been shown by Buckmaster (1917, 1 and 2) to be by far the most important, if not the sole, means of transport of carbon dioxide, that is by *hæmoglobin*. He shows that pure hæmoglobin alone is capable of taking up large quantities of the gas, and giving it off again at a lower tension.

An objection might be made that the complete proof has not been given that these observers were dealing with hæmoglobin itself and not with the sodium salt. In the latter case, the salt would probably be decomposed by carbon dioxide with the formation of sodium bicarbonate and apparent combination with the gas. It is true that Bohr (1887, p. 170) makes the statement that he tested the alkali content of his solutions and found it absent in one case and too small to have any appreciable effect in the other.

Let us first consider the properties of sodium bicarbonate in this respect. Bohr's figures (1909, 2, p. 69) for the dissociation curve of this salt show that at a tension of carbon dioxide of 40 mm. of mercury there is practically no dissociation, the whole of the salt exists as bicarbonate. Even at 12·5 mm. tension, there is only given off 1·9 per cent. of the gas contained in the salt. Now the tension of carbon dioxide in the alveolar air of the lungs is 40 mm. of mercury, so that bicarbonate does not give up any appreciable fraction of its carbon dioxide to this air. The fact that the tension of the gas in the alveolar air is high enough to convert all the alkali of the blood into bicarbonate, is made use of in the method of Van Slyke (see Van Slyke and Cullen, 1917), to determine the amount of the reserve alkali of the blood. Whatever the tension of the carbon dioxide in the tissues may be, none of it taken up by the blood in the form of bicarbonate can be got rid of again merely by exposure to alveolar air. It has been said, however, that the hæmoglobin and other proteins of the blood act as acids and assist in driving off the gas from the bicarbonate. Apart from the fact that it is difficult to conceive how this property does not also prevent the combination of carbon dioxide with the alkali of the plasma, it has been shown by Buckmaster that carbon dioxide comes off from bicarbonate in a vacuum no faster in the presence of hæmoglobin than in its absence. If any protein combines with the sodium, displacing carbon dioxide, it must be because the protein is a stronger acid. If so, the sodium salt of the protein cannot be decomposed by the carbon dioxide of the tissues. The process can occur but once in the life of an animal.

Parsons (1919), on the assumption that proteins (hæmoglobin in particular) act as weak acids competing with carbon dioxide for possession of the available sodium, calculates that the observed experimental data can be satisfied. But there is very good evidence that proteins do not behave as acids at the H ion concentration of blood (Bayliss, 1919, 2). At all events, the experimental proof of the hypothesis in question has not yet been given. Cushny (1920) shows that serum proteins do not combine with sodium or potassium at the H ion concentration of the blood.

Although it may be that the proteins of the plasma take up and give off a small quantity of carbon dioxide, reversibly, owing to adsorption, there is reason

to doubt whether they form salts with any acids but strong ones. The diamino-acids derived from proteins by hydrolysis combine with carbon dioxide, but they are strong bases. Fletcher and Brown (1914) showed that muscle, on heating to 40 degrees, gives off no more carbon dioxide than that driven off from bicarbonate by the lactic acid formed. A further evolution occurs on scalding, which may be due to decomposition of carbamino-proteins (Siegfried), but this clearly is of no importance for our present purpose.

The dissociation curve of hæmoglobin as regards carbon dioxide is given by Bohr (1887). The upper curves of Fig. 196 give that of blood, according to Christiansen, Douglas, and Haldane (1914). Comparing them with the bottom curve, that of sodium bicarbonate in the concentration occurring in blood, accord-

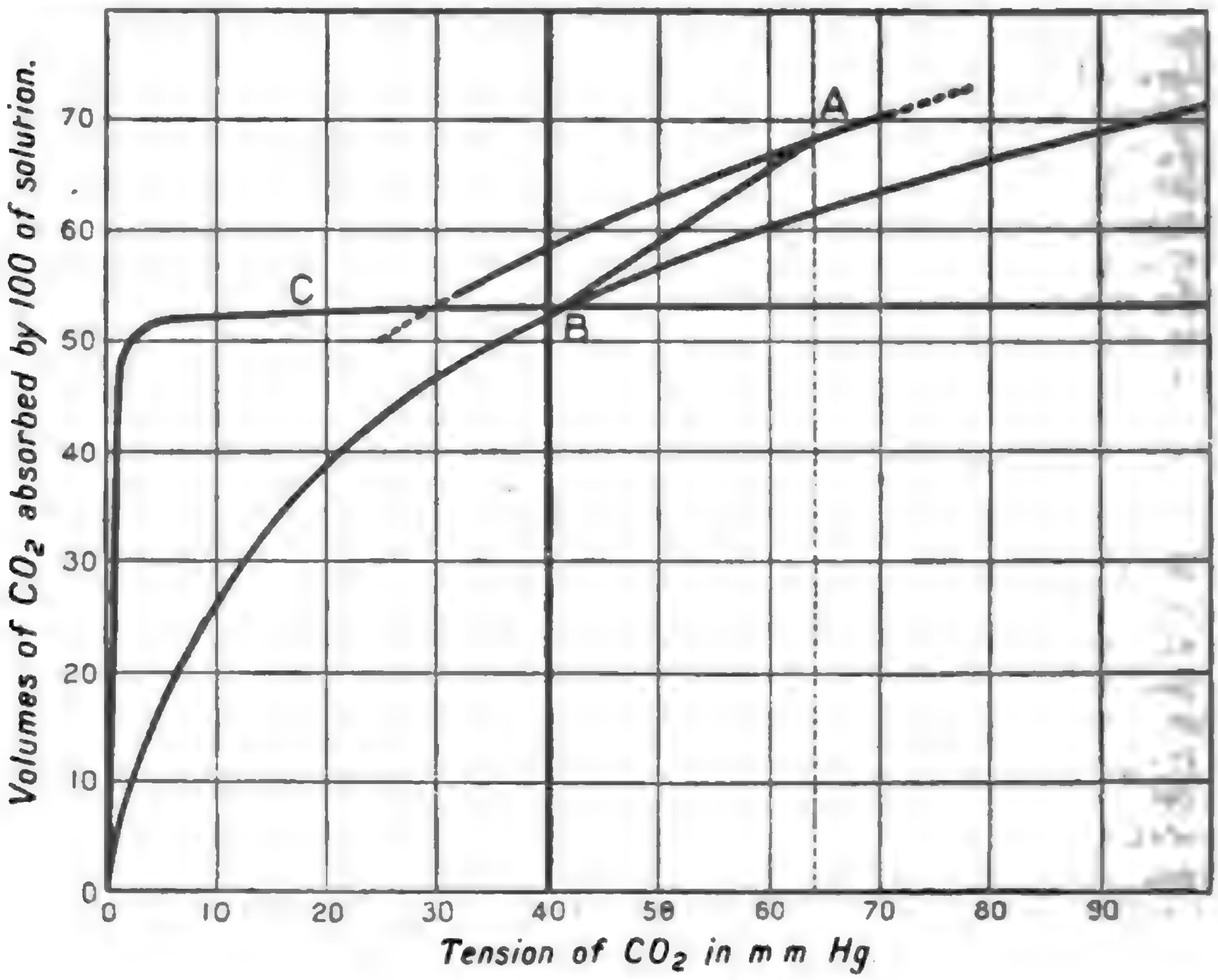


Fig. 196. Carbon Dioxide in Blood.

A, Portion of dissociation curve of carbon dioxide in blood in *absence* of oxygen.
B, Dissociation curve of carbon dioxide in blood in *presence* of oxygen.
C, Dissociation curve of 0·25 per cent. sodium bicarbonate (=concentration in blood). (Bohr.)

Abscissæ—tension of carbon dioxide in mm. of mercury.
Ordinates—volume absorbed by 100 c.c. of the solution.
Thick vertical line—tension of carbon dioxide in alveolar air.
Dotted vertical line—approximate tension of carbon dioxide in the tissues.
Between these lines no carbon dioxide is given off by bicarbonate; whereas venous blood (*A*) gives off carbon dioxide to alveolar air.
Since, at a given tension of carbon dioxide, arterial blood holds less carbon dioxide than does venous blood, the thick oblique line from *A* to *B* represents the volume of carbon dioxide given off in the lungs.
Curves *A* and *B* from Christiansen, Douglas, and Haldane (1914).

ing to Van Slyke, we see how essentially the curves differ. We note that the dissociation curve of sodium bicarbonate consists essentially of two straight lines, a vertical one at the dissociation tension of the salt, a nearly horizontal one from about 10 mm. tension of carbon dioxide onwards. The intersection is rounded off owing to electrolytic dissociation, as pointed out on page 618 above. The thick vertical line is at the value of the carbon dioxide tension in the alveoli, the dotted one at the approximate tension in the tissues. The dissociation in the lungs must be between these limits, and the bicarbonate curve is horizontal here and can play no part. The blood curve (hæmoglobin), on the other hand, shows that carbon dioxide can be given off by it.

The curves given by Parsons (1919, p. 57) show the same fact, but I have not used his figures since they are based on the assumption that all the carbon dioxide in the blood is present as sodium bicarbonate, which is the question at issue.

It appears that we must regard hæmoglobin as being capable of taking up a variety of gases of different chemical nature, and that the relative amounts taken up from a mixture are subject to definite laws (see page 622 above).

The function of *sodium bicarbonate* is that discussed on pages 199-201, namely, to stabilise the hydrogen ion concentration of the blood, not to act as a carrier of carbon dioxide for respiratory purposes.

So far as we know at present, it appears that *proteins* other than hæmoglobin may transport a small amount of carbon dioxide, but that it is of subordinate importance.

The controversy respecting the carriage of carbon dioxide by the blood gives an opportunity of calling attention to a point of general interest in the explanation of physiological phenomena. It may be looked at from several aspects. While there is no doubt that many experimental results can be satisfactorily reproduced by a sufficient number of mass action equations with appropriate constants, it does not follow, without actual evidence, that such is the mechanism of the process. In the doctrine of energetics we have to consider the factor of potential as well as that of quantity. There are, on the chemical side, reactions which occur suddenly when a particular potential is reached, such as the calcium carbonate system, or sodium bicarbonate, which do not give off carbon dioxide until the tension of this gas is reduced to a certain value, and at this point there is a more or less complete and rapid dissociation, obscured to some extent in solutions by electrolytic dissociation. Again, there is the dissociation of strong electrolytes, which does not obey the ordinary law of mass action, and we may also call to mind the various ways in which "activity" coefficients have to be introduced into equations of osmotic pressure, solubility, and so on. Finally, there is decomposition by electrolysis, which does not occur until a definite electromotive force is applied. Other cases will come to the reader's memory. We may say that there are numerous systems in which there is not a gradual transition from one state to another, but two different systems above and below a particular point.

SUMMARY

The object of the respiratory mechanism is to provide for a supply of oxygen to the tissues of the larger animals, where direct access is impossible, and for the escape of the carbon dioxide formed in combustion.

The oxygen has to be taken up from the air and conveyed to the tissues in solution in a liquid, the blood. In the tracheate insects, the gas is supplied directly to the tissues by means of fine tubes containing air.

The necessity of continued supply of fresh air was proved by Robert Hooke in 1667, and the essential constituent, oxygen, was discovered by John Mayow in 1674. Black, in 1755, showed that the product of combustion in animals is different from common air and called it "fixed air"; its nature as an oxide of carbon was discovered by Lavoisier in 1775.

There is no evidence that oxygen is stored in the cells in an "intra-molecular" or other form available for oxidation, with the exception of the very minute amount present as peroxide. Such conceptions as that of "biogen molecules" are not in agreement with experimental facts.

The phenomena of narcosis are not due to inhibition of oxidation, but to changes in the properties of the cell membrane.

Consideration of "life without oxygen" leads to the view that the actual source of the free energy required by a living organism is a secondary matter. If it cannot be obtained by oxidation, other chemical reactions, although of a less efficient kind, are made use of.

Data of the actual consumption of oxygen by various tissues are given in the text. The heart is found to use oxygen in direct proportion to the tension energy developed. There is no more consumption of oxygen by the lungs themselves than by other organs in rest; that is, there is no evidence of oxidation of metabolic products from other organs and contained in the blood. The actual amount of oxygen consumed in the lung tissue is only about half that consumed by the salivary gland at rest. The blood itself in mammals only consumes minimal amounts of oxygen, except when large numbers of young cells are present, as after anæmia. Nucleated red blood corpuscles consume considerable amounts of oxygen.

Since the supply of oxygen required is much greater than could be carried in ordinary solution, there is a special substance, hæmoglobin, in the red corpuscles, which has the remarkable property of taking up oxygen in amount proportional to the pressure of the gas. Thus it takes up oxygen in the lungs and passes it on to the tissues where the oxygen tension is low.

Hæmoglobin contains iron, and it is held by many that the oxygen taken up is, in some way, in combination with the iron, since it appears to be in molecular proportion to it.

There is no chemical system known which has properties like those of the oxygen-hæmoglobin system. Hence it seems probable that surface phenomena act as controlling factors in the amount of the "compound" present at a given oxygen tension. But no satisfactory explanation has been as yet suggested.

The amount of oxygen which hæmoglobin can take up at a given tension is lowered by rise of temperature and by the presence of neutral salts or acid. The importance of these facts with regard to its function is pointed out in the text.

A brief account of the phase rule is given in the text, in connection with the possibility of its application to the case of hæmoglobin.

It is found that an exponential formula expresses the relation of hæmoglobin to oxygen in presence of salts or acid. The difficulty of interpreting this formula in terms of mass action is pointed out. The intervention of phenomena of aggregation are suggested by the fact that hæmoglobin behaves as if in colloidal solution.

Organs, lungs, or gills are provided by which a large surface of blood is exposed to the medium which contains oxygen, that is, to the air or water. Mechanical means of periodic change of the medium ensure efficient oxygenation, together with the escape of carbon dioxide.

The question as to whether sufficient oxygen can be taken up by mere diffusion is discussed. It is found that the only results which cannot, as yet, be explained thus are those of Douglas, Haldane, and their co-workers on the condition attained after some days' acclimatisation to high altitudes. Even in vigorous muscular work there is no need to assume special oxygen secreting power on the part of the cells of the alveoli of the lungs.

The amount of air pumped in and out of the lungs is regulated by the action on the respiratory centre of the hydrogen ion content of the arterial blood. This concentration in hydrogen ions is determined, under ordinary conditions, by the carbon dioxide tension in the alveolar air of the lungs. Under special conditions, as in acclimatisation to low barometric pressures or by feeding on substances which increase the acidity of the urine and blood, other acids, non-volatile, formed in tissue metabolism, assist in the stimulation of the centre

There seems to be no evidence that the excitability of the respiratory centre to carbon dioxide is sensibly affected by want of oxygen, until the oxygen tension has become very low.

In asphyxia, at a certain stage, products of disintegration of the cells of the nerve centres themselves act as exciting agents on these centres. But these products are not to be regarded as normal stimulants.

The function of the reflex nervous mechanism, whose afferent fibres are contained in the vagus nerves, is to regulate the rate of respiration. An expansion of the lungs, resulting from stimulation of the centre by carbon dioxide, is cut short by the inhibiting reflex from the vagus endings in the lungs, and the centre prepared for another stimulation by carbon dioxide. The reflexes are subject to the laws of double reciprocal innervation.

A short account is given of the phenomena of mountain sickness, due to want of oxygen only. "Acapnia," or want of carbon dioxide, plays no part in them.

The transport of carbon dioxide from the tissues to the alveolar air of the lungs is probably effected by means of hæmoglobin, in a way similar to that in which it conveys oxygen. Bicarbonates do not give off gas to a tension of carbon dioxide such as exists in the alveolar air. Whether proteins other than hæmoglobin assist in the process is doubtful; if they do so, it must be by some adsorption process and is not great.

LITERATURE

General.

Babák (1912). Haldane and Priestley (1905). Winterstein (1912, 2). Douglas (1914). Krogh (1916). Head (1889).

CHAPTER XXII

ELECTRICAL CHANGES IN TISSUES

We have already had occasion to refer to the electrical changes occurring in nerves, muscles, and glands when excited to activity. With the exception of the electric fish, these responses are chiefly of interest on account of the light they throw on the physiological processes themselves, and they frequently serve as a valuable means of investigating these processes. Perhaps the most striking of these cases is the use now made of the string galvanometer in researches on the heart, both in health and in disease.

At the time when physiological phenomena began to be systematically worked at from the point of view of accurate measurements, the electrical phenomena naturally attracted much attention, on account of the methods available for the precise determination of the value of electrical currents, and much valuable work was done at that time.

METHODS OF INVESTIGATION

Since so much depends on the instruments used, it is worth while to give some attention to the principles involved in the construction and use of these instruments. Many of these principles will be found to apply to the methods used in other investigations, such as the measurement of sudden changes of pressure, as in the heart beat.

The two factors of electrical energy, capacity and intensity, lead to the division of the instruments used into two main classes; those for the measurement of current, galvanometers, and those for the measurement of potential, electrometers. But, as we shall see, the galvanometers used in work on the electrical changes of tissues allow, as a rule, so small a current to pass that they behave practically as electrometers, and the indications given by the two kinds of instrument are very much the same. The reason why the galvanometers have so high a resistance is, of course, on account of the very high resistance of the external circuit, the tissues, and the galvanometer gives the largest deflection when its resistance is equal to that of the outer circuit.

Galvanometers.—All of these depend on the relative movement of a magnet and a wire through which a current flows. In one type, such as that known as the Kelvin form, the magnet moves under the influence of a current in a wire surrounding it; in the other type the magnet is stationary, and may be either a permanent magnet, as in the D'Arsonval pattern and many forms of commercial ammeters and voltmeters; or it may be an electro-magnet, as in the string galvanometer of Einthoven. In this second type, the wire conveying the current moves. In the D'Arsonval instruments the wire is in the form of a light rectangular coil; in the Einthoven form it consists merely of a very fine wire stretched between the poles of a powerful electro-magnet. When a current passes through the wire of the string galvanometer, the wire is deflected to one side or the other, according to the direction of the current, and the movement is magnified by a microscope and photographed by projection on to a slit, behind which is a moving sensitive surface.

Inertia of Moving Parts.—When an electrical change lasts a very short time, it is plain that it would be able to move a very light system when it was unable

to make any impression on a heavier one. Just as a slight impulse would not cause a visible movement of a cannon ball, but might give considerable motion to a pith ball. This is especially to be taken account of when the electrical change is subject to rapid alterations. The moment of inertia of the moving parts should, therefore, be as small as possible.

Damping.—Suppose that a short-lasting electrical current has set one of these systems into movement. It is clear that, unless there is some influence to bring it to rest, the movement will continue after the current ceases. Now our object is to obtain as true as possible a record of the time course of an electrical effect. When a wire forming part of an electrical circuit moves in a magnetic field, a current is developed in it in such a direction as to oppose further movement. This will occur whether the wire is conveying a current already or not. In the D'Arsonval and the string galvanometers, therefore, as long as the circuit is closed, any movement of the wire produces in it a current tending to stop its movement. The extent of the opposing current depends, by Ohm's law, on the resistance of the circuit, hence also the damping effect. In physiological work this resistance is very large, hence the damping is not any greater than required. In fact, in the D'Arsonval instruments, it is usually necessary to add a short circuit to increase the damping; in the beautiful galvanometer of Moll (1913), made by Giltay of Delft, the damping is regulated by altering the strength of the current producing the magnetic field. In the Kelvin instrument, the damping is usually effected by air resistance to the movement of a vane on the magnet system. When the moving system is heavy, the vane is sometimes made to move in a bath of oil.

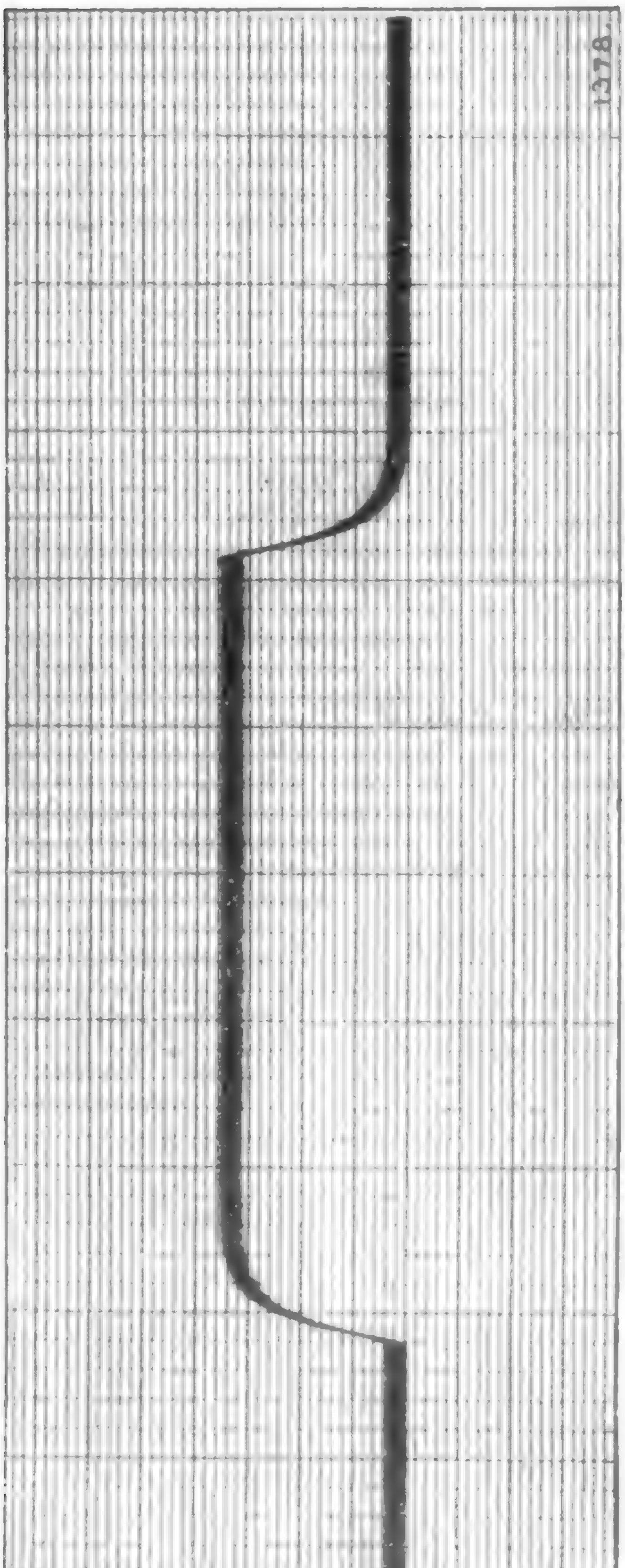


Fig. 197. The string galvanometer.—Deflections produced by make and break of a current of 2×10^{-8} amperes. Slight tension on fibre.

Period of Vibration.—Since the moving system must be brought back to its resting position by some force, such as the magnetism of the earth or the torsion of a wire, there will be oscillations similar to those of a pendulum. These would prevent the real value of a deflection from being estimated, so that, except for special purposes, the movement is made as nearly as possible "aperiodic" by appropriate damping. When this is the case, the deflection is reached without vibration around it. This is associated, however, with a

slower rate of movement, so that, as a rule, some compromise has to be made. In any case, the damping should not exceed that necessary for aperiodic movement. To illustrate the point, Figs. 197 and 198 may be consulted. It will be seen that the deflection is aperiodic in both cases, but with a slight tension on the string the deflection is as large with a small current as that with a larger current if the string is tighter. But the rate of movement, or time taken to reach the final position, is more rapid with the tighter string. If rapid changes are to be followed correctly, therefore, the string must be tight enough to move as quickly as the electrical effect to be observed. Fig. 199 (from the book by T. Lewis, 1913) shows the different rate of movement of the string under different tensions. If too slack, it does not follow quick changes, like the first ventricular phase of the heart, with sufficient rapidity to give their full value.

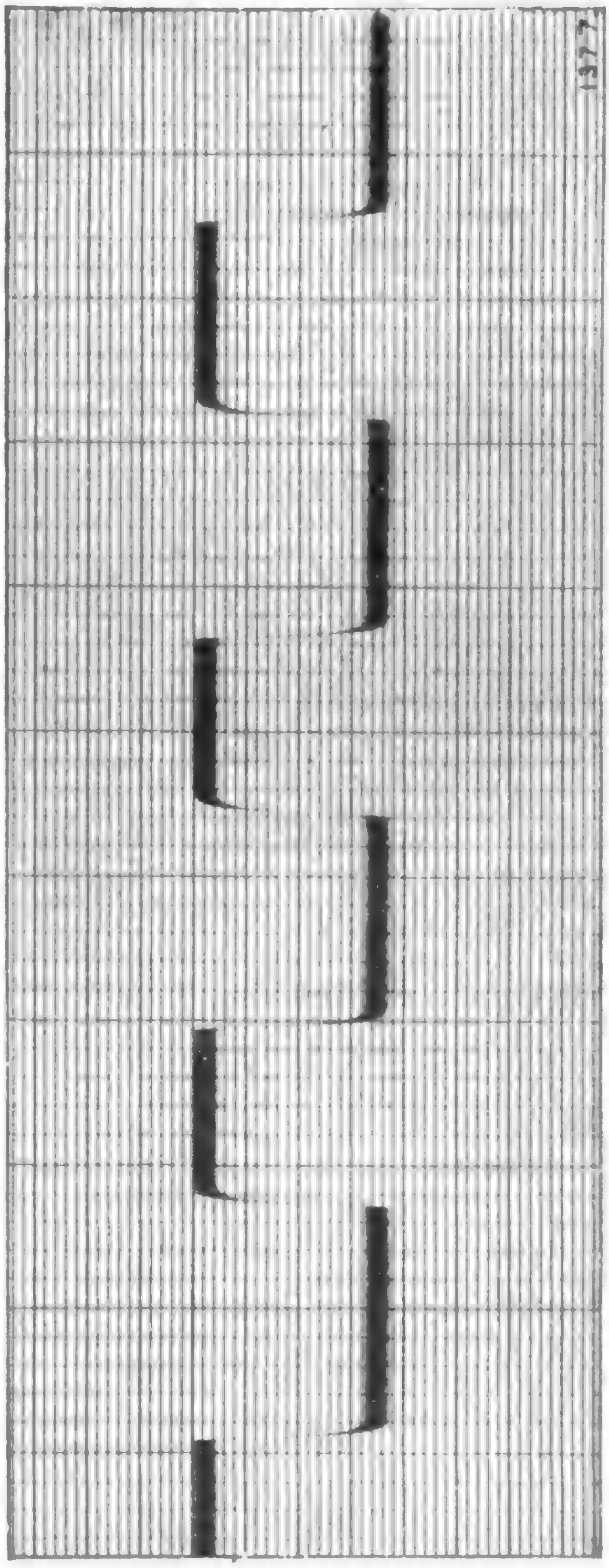


FIG. 198. Similar curve with increased tension on fibre and current of 10^{-8} (increased on account of less sensitiveness of the tighter fibre).

Note that full deflection is reached in shorter time in this case than in that of the preceding figure. But in both cases the movement is aperiodic.

Time interval between vertical lines in both figures, 0·04 sec.

(Camb. Sci. Instrument Co. Galvanometer, Catalogue No. 126, p. 30.)

Figure of Merit.—The sensitivity may be considered to be the deflection produced by a given current. To compare different galvanometers, the period of vibration and also the resistance should be taken into account, so that we obtain what is known as the "figure of merit" (E).

$$E = \frac{100 \times D}{T^2 (R)^{\frac{1}{2}}},$$

or, $$= \frac{100 \times D_1 \times R^{\frac{1}{2}}}{T^2}.$$

where T is the periodic time in seconds,
R is the resistance of the galvanometer in ohms,
D is the deflection in mm. for one microampere at one metre,
D_1 is the deflection in mm. for one microvolt at one metre.

For the properties to be taken into account in choosing a galvanometer for a particular purpose, the catalogue of the Cambridge Scientific Instrument

Company may be consulted. In general, if the deflection required is to be as large as possible, or a very small electrical current is to be detected, the Paschen form of the Kelvin instrument is the best. If the time relations of a complex change are to be ascertained by photographic record, the "*string*" *galvanometer* of Einthoven is to be used. This latter form is that with which most work is now done. Fig. 200 represents the instrument as made by the Cambridge Instrument Company. A camera with moving plate or paper is used with it, and an arc lamp for illumination. The shadow of the string is photographed. A diagram of the mechanism is given in Fig. 201.

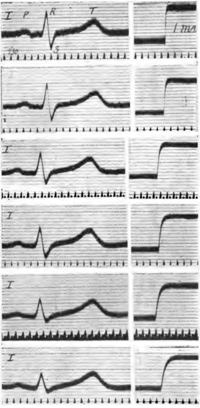


Fig. 199. Electro-cardiograms of the human heart, taken with different tensions of the fibre of the string galvanometer. The tensions diminish in the series from above downwards. Led off from right arm and left leg. The curve to the right of each figure shows the time taken to attain full deflection when one millivolt is applied. This time increases as the tension of the string diminishes, although the movement is aperiodic in all the cases given. It will be noticed that the rapid changes in R and S are not correctly followed with low tension, but that the slower rate of change in T is equally well given in all.

(Lewis, 1913, 1, p. 10.)

In the *Oscillograph* of Duddell, the movement of the system follows changes of current rapidly by the fact that its own vibration period is enormously high compared with the rate of change of the current to be investigated. This vibration rate may amount to 10,000 per second. The same principle is used for the correct registration of the pressure changes in the heart and the blood vessels. The oscillograph is practically a moving coil galvanometer, with the coil reduced to a thin loop of stretched phosphor-bronze. It is used chiefly for the investigation of the wave form of alternating currents.

Electrometers.—Although there are several forms of instruments used in physical work for measurement of potential, the *capillary electrometer* of Lippmann is practically the only one used in physiology. It consists of a slightly conical capillary tube, containing mercury, whose point is immersed in 20 per cent. sulphuric acid. The mercury is forced into the capillary tube by pressure until the meniscus of its contact with the acid is at a convenient place. One electrode is connected with the mercury in the tube, the other with a mass of mercury also in contact with the acid. Fig. 202 shows one pattern of the instrument. When connected to two points at different potentials, so that that which is positive is in connection with the mercury in the capillary, the surface tension at the contact with the sulphuric acid is diminished, and the mercury is forced by the pressure

on it, which kept it in its place in the tube, further towards the point. If negative, the surface tension is increased and meniscus retreats, since its surface tension, which was previously balanced by a given external pressure, will now only be balanced by this same pressure at a wider part of the tube. Until the mercury has come to rest, a certain charge of electricity flows into the instrument, which behaves as a condenser. After this no current flows. The movement may be very rapid and is completely aperiodic. The contact surfaces of the acid and mercury are polarised, but the theory is somewhat complex, and may be found in Freundlich's book (1909, pp. 184-212). Roughly speaking, it may be said that when the mercury meniscus is positive it becomes oxidised and dirty, when negative it is reduced to bright metal. Hence the surface tension is low in the former case, high in the latter. The movements are photographed by projection on a slit, behind which a photographic plate is moved. The curves obtained require correction, but the law governing the position of the meniscus at any time after connection with a source of electromotive force is a definite one and readily determined for each instrument (see the papers by Burch, 1890, 1892, and by Keith Lucas, 1909, 2). The curve is a logarithmic or exponential one, with the equation :—

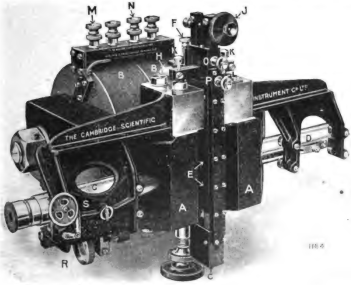


Fig. 200. General appearance of the string galvanometer, as made by the Cambridge Scientific Instrument Co., in accordance with the pattern designed by Einthoven.

A, Poles of electro-magnet, itself excited by current through coils *BB*.
C, The fibre case, containing the stretched "string."
G, *D*, Microscope and condenser, passing through holes in the pole pieces.
E, Narrow air gap between poles, containing the string.
J, Adjustment for tension of string.
H, Screw for centring the string case, which rocks on the two screws *K*.
R, Fine adjustment of microscope.

(Cambridge Sci. Instr. Co., Catalogue No. 126, p. 23.)

$$\log_e \frac{y}{a} = -ct,$$

or,

$$y = ae^{-ct},$$

where y is the ordinate of any point P measured downwards from the asymptote, that is, the level at which the meniscus finally comes to rest, t is the horizontal distance of P from a point on the asymptote taken as origin of co-ordinates (that is, time from commencement of charge), a and c are constants, e is the base of natural logarithms (see Fig. 203). It will be seen, on reference to the curve, that the potential difference between the terminals can be determined by taking any point on the curve, without the necessity of its having arrived at the limit of its movement; in fact, it may be brought back at any point in its course by the application of an opposite potential difference, without interfering with the measurement of that which produced the original movement. The potential difference causing any deflection may be considered as made up of two parts, one represented by the ordinate of the curve at any time, and the other represented by the vertical distance the meniscus has still to move. The latter is a function of the rate of movement at the time taken, that is, of the steepness of the curve. It is, therefore, measured by the angle made by the geometrical tangent of the curve with the axis of abscissæ. The simplest way of analysing a complex curve, obtained experimentally, is that of Keith Lucas (1909, 2, p. 218). Each tube has, of course, to be calibrated, the rate of movement depending partly on the electrical resistance, partly on mechanical resistance to movement, probably friction. The movement is quite aperiodic. The method of drawing tubes will be found in the paper quoted.

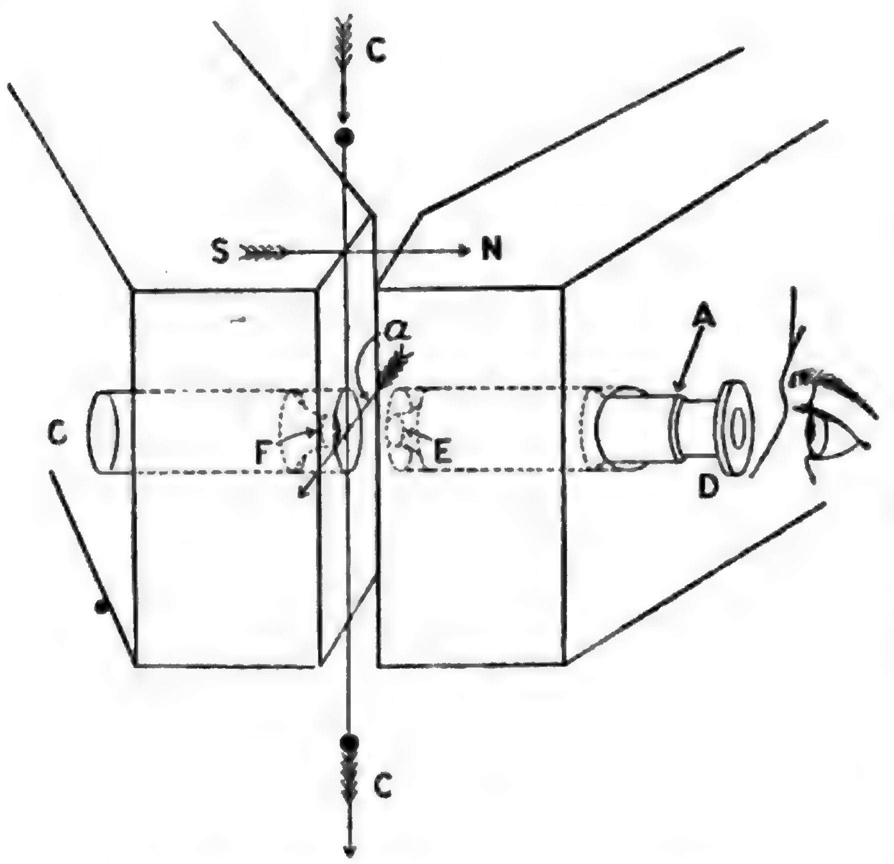


FIG. 201. DIAGRAM OF MECHANISM OF STRING GALVANOMETER.

The fine wire ("string"), *CC*, is stretched in the narrow gap between the poles *N* and *S* of a powerful electro-magnet. When a current passes through the "string" in the direction of the vertical arrows, the wire is deflected in the direction of the arrow *a*, that is, at right angles to the magnetic field *NS*. This small movement is observed, or projected on to a photographic plate, by means of a microscope, *ED*, the light of an arc lamp being condensed by the lens *F* on to the string.

This electrometer, although sensitive enough for most work, is less so than the string galvanometer, but, for exact analysis, the photographed records of the former present certain advantages in that the analysis is simpler, following a better known law than those of the string galvanometer (see the paper by Keith Lucas, 1909, 2, p. 210).

Other forms of electrometer have been little used in physiological work, although it seems possible that the *string electrometer*, in which the string moves between plates with opposite charges, will be found useful (for a description of the instrument, see the Cambridge Instrument Co.'s Catalogue of Electrometers).

The Circuit.—The arrangement of the connections is practically identical with that used in the measurement of the electromotive force of a concentration battery (page 192). The diagram of Fig. 204 may be found useful.

The necessity for the use of non-polarisable electrodes, even for such brief currents as those from the heart, is shown by the photographs given by Thos. Lewis (1915, 3).

Rheotomes.—The repeating rheotome, by which corresponding bits are cut out, as it were, from a series of electrical responses by means of contacts arranged to be made and broken by a rotating wheel, is rarely used at the present time. The introduction of more accurate and sensitive instruments and means of analysis of photographic curves has practically displaced

it. The use of a device for opening a series of keys at known short intervals of time after one another by a pendulum is frequently indispensable, and the most convenient way of doing this is by the instrument described by Keith Lucas (1908, 2).

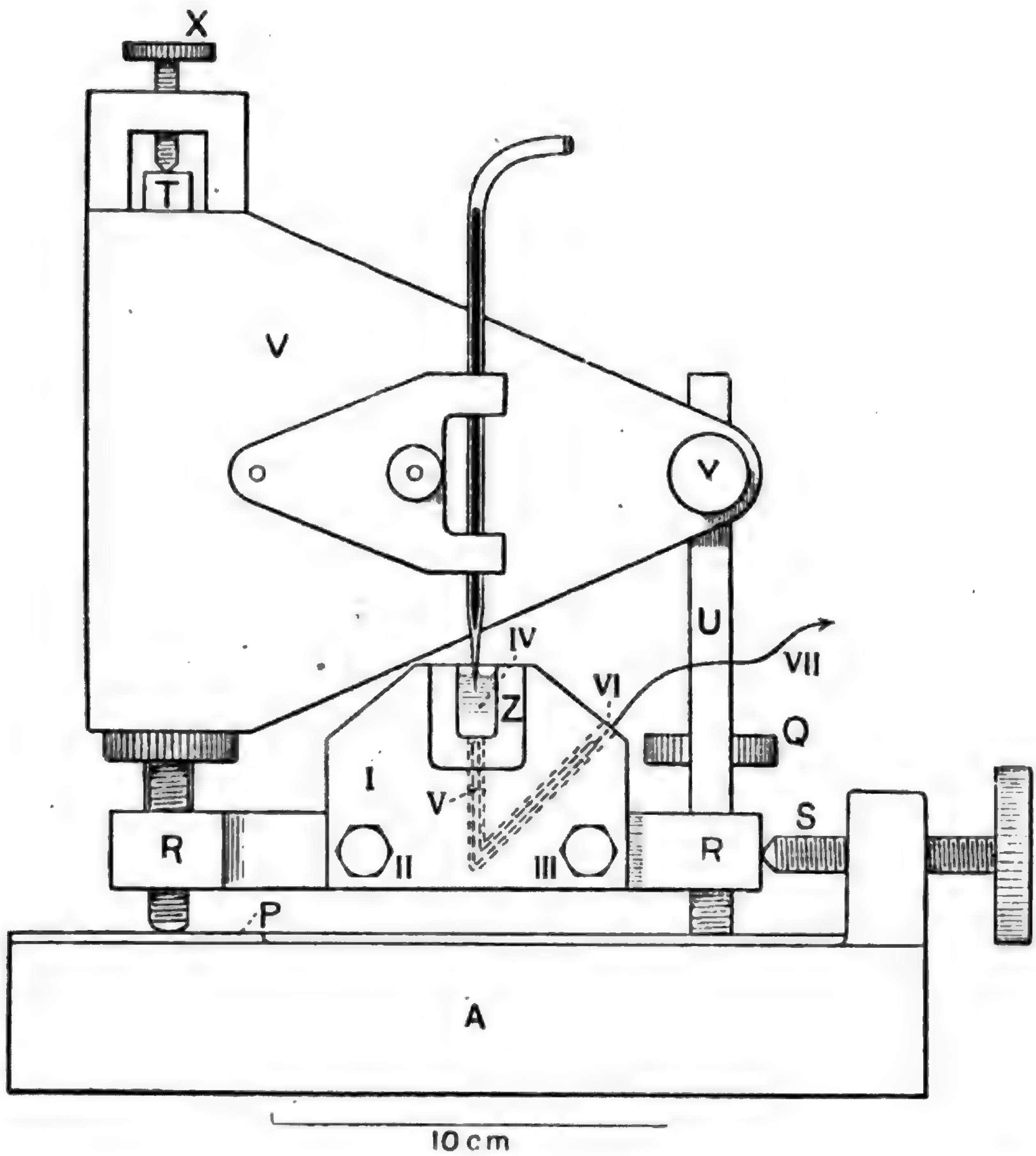


FIG. 202. CAPILLARY ELECTROMETER.—Keith Lucas' pattern. End elevation. The projecting microscope is supposed to be perpendicular to the plane of the paper.

A, Heavy base.
R, Casting on levelling screws *Q*.
S, Screw for lateral adjustment.
T and *U*, Upright rods, supporting ebonite plate *V*.
X, Screw for vertical adjustment.
Y, Screw for moving capillary at right angles to plane of paper.
I, Block of ebonite, attached to *R* by bolts *II* and *III*.
IV, Notch cut out to contain acid, closed at the sides by cover-glasses *Z*, cemented by hot gutta-percha.
V and *VI*, Two holes, meeting at the bottom, containing mercury, into which dips the platinum wire *VII*.

(Keith Lucas, 1909, 2, p. 212.)

ORIGIN OF POTENTIAL DIFFERENCES IN TISSUES

That the electrical phenomena observed in the activity of cells are due to changes of potential, and not merely to changes of resistance, is evident, not only from the fact that they are shown by instruments, electrometers, which do not respond to changes of current only, but also in the ordinary way of demonstrating

these electrical responses by means of a sensitive galvanometer. In doing this, any current already existing in the circuit is balanced by an opposing potential difference from the slide wire, in order to bring the deflection to zero before stimulating the tissue. No mere change of resistance can, in such circumstances, cause a deflection; there is no potential difference to create a current.

Let us next examine the *possible sources* of potential difference in living tissue.

We may take it that these potential differences must be due to electric charges on ions. It does not seem probable that phenomena of frictional electricity play any part. In any case, these phenomena themselves can generally be traced to the production of some kind of ions. The question was discussed to some extent in connection with the charge on colloidal particles (page 89).

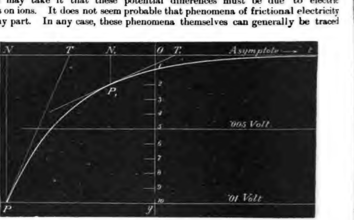


FIG. 203. NORMAL CURVE OF CAPILLARY ELECTROMETER.

Produced by applying a potential difference of 0·01 volt between its terminals at the point P of the tracing. Considerable resistance was introduced into the circuit, so that the rate of movement is slowed.

Ordinates—position of meniscus. The divisions along the axis Oy are in 0·001 volt.

Abscissæ—time. Ot is the axis and also the asymptote of the curve. The portion of the curve above the line through 5 is the entire normal curve for 0·005 volt, and the portion above P_1 is that for 0·00165 volt.

PN and P_1N_1—Ordinates at P and P_1.

PT and P_1T_1—Tangents to the curve at P and P_1.

NT and N_1T_1—Subtangents. These are equal to one another.

The equation to the curve is

$$\log \frac{y}{a} = ct,$$

where y is the vertical distance of any point from the asymptote of the curve, and t is the horizontal distance from the origin of abscissæ. a and c are constants of a particular capillary tube.

(Burch, 1890, p. 91.)

The part played by electric charges on surfaces is treated of in the paper by Mines (1912, 1).

That the source is ionic is indicated by the *temperature coefficient* of the electromotive force of tissues. This was determined by Lesser (1907), for the skin of the frog, and found to be proportional to the absolute temperature. If the source were some kind of chemical reaction, a much higher temperature coefficient would be found.

If the ions arising from electrolytic dissociation of a substance are free to move and intermix, it is clear that no potential difference could be detected. Moreover, the increase of their numbers, whether by increased dissociation or by production of new ones by the splitting up of larger molecules, or by the setting free from a state of adsorption, in itself cannot give rise to any change in potential difference,

except a very temporary one. Unequal rate of diffusion from their place of origin is the cause of this temporary electrical state.

Suppose that the ions, newly produced, are free to diffuse. As we have seen

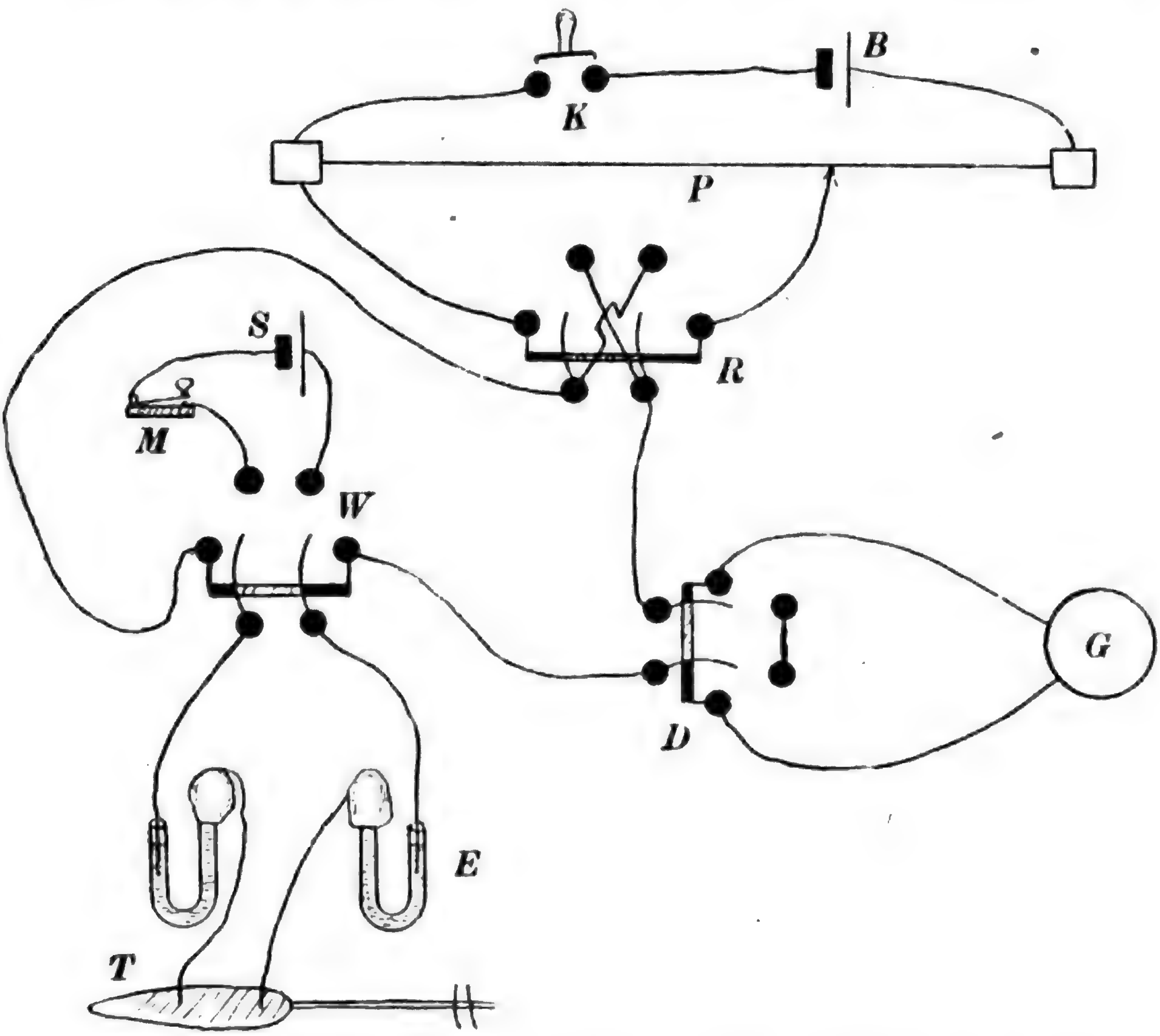


Fig. 204. Diagram of circuit used in observations and measurements of changes in difference of electrical potential.

B, Half-discharged accumulator cell, sending constant current through the stretched wire *P*, from which various fractions can be led off by the sliding contact.
K, Key for this circuit.
R, Reverser, or commutator, by which the direction of the electromotive force led off can be changed without changing the connections of the battery.
S, Standard cell, which can be put into the circuit by the key *W*.
M, Spring key for momentary closure, in order not to take off any appreciable current from the standard cell.
G, The instrument used for detecting the electrical disturbance, electrometer or galvanometer. It can be short-circuited by turning the switch *D* into the position opposite to that shown. When the capillary electrometer is used, a key should be introduced which keeps the instrument always short-circuited except when in use.
E, Nonpolarisable electrodes, leading off from points on the muscle *T*.
E and *T* together represent any source of potential difference, such as those in tissues or in a hydrogen electrode.

(page 158), the more rapidly moving ions will proceed in advance of the slower ones, giving rise to a potential difference in proportion to the difference of their velocities. In the small spaces within cells through which this diffusion takes place, any difference of concentration will equalise itself very rapidly, and, except in the case of the hydrogen and hydroxyl ions, can never give rise to any considerable electromotive force. The factor expressing this occurs in the formula

for the electromotive force of concentration batteries (see Nernst's book, 1911, p. 752). It is

$$\frac{u-v}{u+v} \text{RT} \log_e \frac{c_1}{c_2}$$

which gives the electromotive force at the contact of two solutions of concentrations c_1 and c_2, u and v being the velocities of the cation and anion respectively. When u and v are very nearly equal, as in potassium chloride, the potential difference due to this factor is very small. If u is hydrogen, with a molecular conductivity of 318, and v is carboxyl, as in formic acid, with a molecular conductivity of 33·7, the fraction $\frac{u-v}{u+v}$ becomes—

$$\frac{318-33{\cdot}7}{318+33{\cdot}7} = \frac{284{\cdot}3}{351{\cdot}7} = 0{\cdot}81.$$

R is $0{\cdot}861 \times 10^{-4}$ and if we take ordinary logarithms and a ratio of concentration of 1 to 10, the expression becomes about 0·05 volt, and even this could only last an infinitesimally short time owing to rapid diffusion.

We see that, to account for the values actually obtained experimentally in animal tissues, another source of potential difference must be found. The potentials of metallic electrodes naturally suggest themselves, so that one sometimes finds it stated that the electromotive activity of tissues is that of a concentration battery. But it is plain that there is nothing in living cells that could be taken as a metallic electrode. On the other hand, we have already seen (page 161) how we can obtain a permanent potential difference of fairly considerable amount when a membrane is present permeable to one only of the two ions of a binary electrolyte. There is formed a Helmholtz double layer, and the electromotive force is expressed by a formula similar to that of the concentration battery. This point of view was taken by Bernstein (1902), on the basis of Ostwald's (1890) considerations regarding semipermeable membranes, and is developed further by Bernstein in a paper of 1913.

It may enable this important conception to be grasped more easily if a simple illustration be given. Imagine two large pastures separated by a fence and that the spaces between the bars of the fence are wide enough to allow lambs to pass through, but too narrow for ewes. Introduce into one of these pastures a flock of sheep, each ewe with one lamb. In the course of their wanderings they will arrive at the fence. The propensity of the lambs to wander further will take them through the fence, while the ewes will be left behind. But the attractive forces, particularly that of food, will prevent the lambs from departing from their mothers for any considerable distance. Similarly, the presence of the lambs in the adjoining field will prevent the ewes from wandering far from the fence. Regarding wool as electric charge, we see that the potential will be higher on the side of the fence occupied by the ewes. It may be said that the thickness of the layer would be considerable, but if we imagine molecules magnified to the size of sheep, the arrangement would not greatly differ from the molecular one.

Since the question is somewhat fundamental, it is well to consider the mathematical proof that a membrane of the kind postulated gives rise to a potential difference expressed by a formula similar to that of Nernst's concentration battery with metallic electrodes.

There are two distinct ways in which the calculation can be made. We may take the work done in moving electricity from one solution to the other against electrostatic forces, as in the method used by Nernst, based on Helmholtz's theory of contact potential, and similar to that used on page 33 in calculating the work done in compressing a gas. But for the present purpose, in which we are regarding the phenomenon from the point of view of the potential difference in equilibrium, the following method is more appropriate, besides giving an opportunity for a new aspect of the case. The details of the treatment I owe to Mr W. B. Hardy.

For simplicity, we will consider the membrane as being infinitely thin, which is very nearly true for the cell membrane. Let it be situated, to begin with, between water and a solution containing a salt which is electrolytically dissociated

into the ions B· and S′, and be freely permeable to B·, but impermeable to S′, in a purely mechanical way, as a filter or sieve, for example.

The ions B· tend to pass from solution inside to water outside owing to their osmotic pressure and to this alone. Since ions S′ cannot pass through, in order that ions B· shall diffuse into the outer solvent, they must separate from their companions. This they cannot do for more than a minute distance, owing to the enormous electrostatic force between the oppositely charged ions. The amount of this force was calculated by Arrhenius, as we saw on page 179 above.

These ions B· are, therefore, acted on by two forces in opposite directions, and they will take up a position in which the two forces are equal and opposite.

The *osmotic pressure* exerted on a membrane of area A is AdP, where P is the pressure per unit area.

The opposite *electrostatic force* is obtained thus:—

Let E be the potential difference between the two members S′ and B· of the Helmholtz double layer. Then $\frac{dE}{dx}$ is the potential gradient, or rate of fall of potential across the space between the two layers.

Further, if q is the quantity of electricity carried by one gram-equivalent of ion B·, then the force acting on this gram-equivalent is $q\frac{dE}{dx}$. That this is so will be clear by consideration of the fact that the force is directly proportional to the quantity of electricity producing it, and the fact that the greater the difference of potential between the layers of B· and S′, the greater will be the attractive force between them.

Let c be the concentration of the diffusible ion B· in gram-equivalents per c.c. of solution.

The volume of the space between the two layers is Aδx, if the depth be δx, and the number of gram-equivalents of the ion B· contained in the space is A$\delta x c$.

Then the force acting upon them, due to the potential gradient $\frac{dE}{dx}$, is $A\delta x c\frac{dE}{dx}q$.

This force is equal and opposite to their osmotic pressure, therefore—

$$A\delta x c\frac{dE}{dx}q = A dP,$$

or,

$$\frac{dE}{dx} = \frac{1}{cq}\cdot\frac{dP}{\delta x}.$$

P is equal to cRT, since $c = \frac{1}{v}$,

therefore,

$$\frac{dE}{dx} = \frac{RT}{Pq}\cdot\frac{dP}{\delta x}, \text{ since } \frac{1}{cq} = \frac{RT}{cRTq},$$

and,

$$dE = \frac{RT}{q}\cdot\left(\frac{dx}{P}\cdot\frac{dP}{\delta x}\right).$$

The treatment will be more general if we suppose that the concentration of the ion B· is a positive quantity on both sides the membrane. In that case, we must integrate between the limits, p_1 and p_2, which are the osmotic pressures of the ions B· on the two sides of the membrane.

$\frac{RT}{q}$ is a constant. Therefore,

$$E = \frac{RT}{q}\int_{p_2}^{p_1}\left(\frac{dx}{P}\cdot\frac{dP}{\delta x}\right)$$

$$= \frac{RT}{q}\log_e\frac{p_2}{p_1} \qquad (1)$$

or, if c_2 is the concentration of the stronger solution and c_1 that of the weaker solution,

$$= \frac{RT}{q}\log_e\frac{c_2}{c_1} \qquad (2)$$

c_1 and c_2, of course, refer to concentrations of the ion concerned, that is, the one to which the membrane is permeable.

Although there are certain experimental difficulties in the actual investigation of the question, especially when inorganic electrolytes are dealt with, some measurements which I made with Congo-red (1911, 2, pp. 245-247) gave results in satisfactory agreement with the formula.

If the system is not in osmotic equilibrium, so that there is a flow of solvent through the membrane, the electromotive force would not be given by the formula.

There is an interesting theoretical difficulty, analogous to that involved in electrolytic dissociation already referred to (page 180), which has not yet received satisfactory explanation. We know experimentally that a Helmholtz double layer is formed, owing to the fact that one ion cannot move far from the oppositely charged one. But it is not easy to see why this should be so. Suppose the electrolyte completely dissociated. On the kinetic theory, this means that the period during which any oppositely charged ions come within each other's sphere of influence is negligible, so far as electric stresses are concerned. The work required to separate the ions is therefore accomplished, and further separation should not require any more energy. So that, if such a solution be separated from pure solvent by a membrane permeable to one kind of ion only, these ions should be able to diffuse out freely, since they are already out of the range of influence of the opposite ions. Of course, if they did so there would be a large increase in the free energy of the system, which would infringe the second law of energetics. But if the force uniting the ions is purely electrical, it is difficult to understand why they cannot separate from one another after being dissociated. Larmor (1908) suggested that the energy for dissociation may be drawn from the volume energy of the solvent.

To return to the question of the electromotive force at a membrane. From the mode of its production, it will be seen to be an illustration of the rationale of metallic electrode potentials, if we regard the metallic ions as being free to escape from the surface of the metal, while the oppositely charged mass of metal cannot. Contrary to the contact potential difference of solutions free to diffuse, it is permanent. We may speak of a membrane of the kind described as being *polarised*. This form of expression is sometimes convenient.

In the application of the above theory to the living cell, we see that, if the membrane is *permeable to both ions*, no electromotive force can be present; although if one ion be larger than the other, there might be only a small number of pores permeable to the larger ion, so that, for a considerable time, an electromotive force might exist. If, moreover, the membrane were *impermeable to both ions*, there could be no potential difference, since there would be no possibility of the ions separating to form a double layer.

In short, a membrane previously impermeable to both ions might give rise to an electromotive force if it became permeable to one only, but not if it became permeable to both. Also a membrane, previously permeable to both ions, might become a source of potential difference if it became permeable to one only. Such changes are, no doubt, taking place in the normal activities of the cell.

The manner of origin of these potential differences is essentially the same as the "electrical forces at phase boundaries," discussed by Haber and Klemensiewicz (1909); the phenomena described by Beutner (1912 and 1913) are, no doubt, due to the same facts. Suppose that we have a layer of a liquid, immiscible with water, in contact with a solution in water of an electrolyte, of which one ion is soluble in the non-aqueous phase, the other insoluble. It is clear that the former ions will tend to pass into the non-aqueous liquid, but cannot get beyond the boundary, owing to the other ions being unable to do so. We have again a Helmholtz double layer. Thus Beutner finds a potential difference at the contact surface between a watery solution of potassium thiocyanate and a solution of toluidine thiocyanate in toluidine. This is readily accounted for if the potassium ion is insoluble in toluidine, SCN ion being soluble.

If, in any cell process, ions are newly formed, these will add to the concentration of those of the same sign already present, and increase the potential difference at a membrane of the kind described, as the formula shows.

We have seen (page 161) that the concentrations expressed in the formula

refer to the total concentration of all the diffusible ions of the same sign present since interchange takes place freely at the contact surface. Thus the chemical nature of the ion does not appear to enter into consideration.

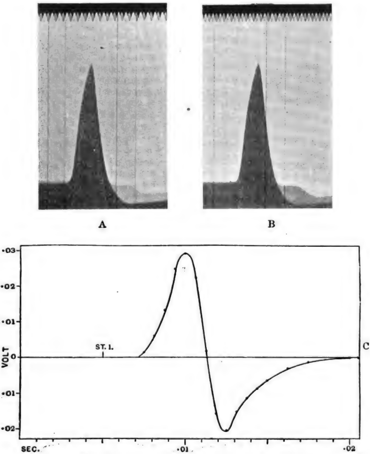


FIG. 205. DIPHASIC ELECTRICAL CHANGE IN UNINJURED SARTORIUS MUSCLE.

A, Photograph of excursion of capillary electrometer. First with a single stimulus. Then, a second time, with an additional stimulus at 0·022 sec. later. The change due to the latter is fainter, because only recorded once. Time in 200 per second.

B, Similar photograph with another additional stimulus at 0·010 sec. after the first, and between it and the second.

In addition to the form of the diphasic response, as recorded by the capillary electrometer, these two figures show that a stimulus, occurring in the refractory period due to a previous one, has no effect on the result of a stimulus given just at the beginning of the return of excitability. That is, a stimulus in the refractory period does not set up any further refractory state.

(Keith Lucas, *Jl. Physiol.*, 43, p. 52.)

C, Analysis of a capillary electrometer record of a diphasic response of the gastrocnemius muscle to a single stimulus applied to the sciatic nerve. Muscle led off from the middle and the tendinous end.

(Keith Lucas, *Jl. Physiol.*, 41, p. 371.)

Charges on the surface of membranes themselves, dealt with by Mines (1912, 1), and discussed in relation to those of colloids on pages 89-91 of the present work, are difficult to bring into relation with the electromotive phenomena of cells. Of course, if a membrane adsorbs preferentially one ion of an electrolyte with which it is in contact, owing to the greater decrease of surface energy by this ion than by the opposite one, the membrane will obtain the charge of the adsorbed ions. Whether this would show itself in the form of a potential difference between the two sides of the membrane seems doubtful.

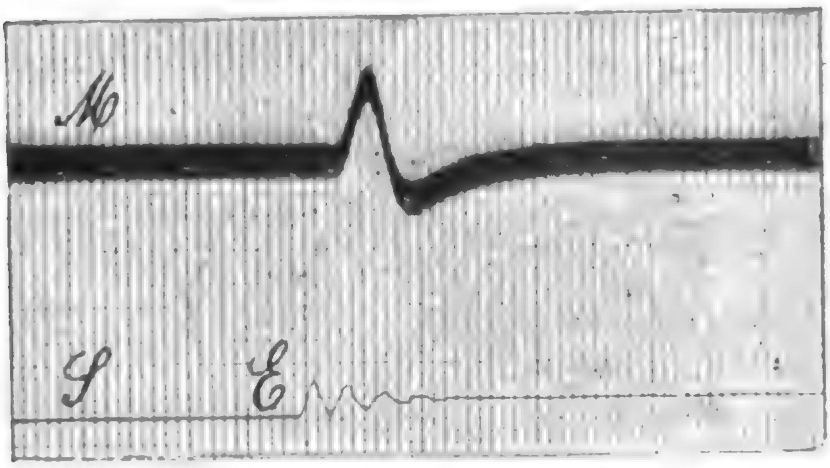


FIG. 206. DIPHASIC ELECTRICAL CHANGE IN GASTROCNEMIUS MUSCLE OF THE FROG.

Led off to string galvanometer from two uninjured places on the surface of the muscle.
Sciatic nerve stimulated by a single shock at *E* on the signal line *S*.
M, The electro-myogram.
One scale division of abscissæ is equivalent to 0·002 sec.
One scale division of ordinates is 7 millivolts.

(Einthoven, 1913, p. 67.)

Baur (1913), however, has described what he calls a model of the electric fish, in which such charges on membranes appear to play a part. If a mixture of "turkey red oil" with three parts of acetylene tetrachloride, be shaken with water and allowed to stand, a separation into an oily phase and a watery phase takes place. The lipoid phase is said to contain some water, sodium sulpho-ricinoleate, sodium sulphate, castor oil, and acetylene tetra-chloride. This is saturated with mercurous sulphate and electrodes made by contact with mercury. Two such electrodes in potassium sulphate solution have, of course, equal and opposite potential differences :—

$$\mathrm{Hg} \mid \text{lipoid} \mid K_2SO_4 \mid \text{lipoid} \mid \mathrm{Hg},$$

so that the combination has no electromotive force. If, however, to the potassium sulphate solution on the one side an electrolyte with a strongly adsorbed cation, such as quinine sulphate, is added, this electrode becomes positive to the other. If the sodium salt of fluorescein, with strongly adsorbed anion, is added, the electrode becomes negative. Thus, with quinine sulphate on one side and fluorescein on the other, an electromotive force of 0·36 volt was obtained. The system is somewhat complex, probably unnecessarily so, and may, perhaps, be equally explicable on the basis of solubility of one ion only in the lipoid phase. The result would be the same.

We may now proceed to refer briefly to some actual cases where electromotive phenomena have been found to accompany the activity of cells.

EXAMPLES

Nerve.—The electrical response in nerve has been discussed above (page 379). Fig. 101 shows its character in the olfactory nerve of the pike.

An important use of the fact has been made in several cases already referred to. The measurements of the time relations of the knee-jerk by Jolly (page 475), the impulses arising in the vagus nerve by distension and collapse of the lung by Einthoven (see Fig. 106, page 386), and the impulses in the depressor fibres caused by increase of pressure in the aorta, also by Einthoven (in Fig. 106), may be mentioned.

Keith Lucas (1912) examines the evidence that has been brought forward to show that the process of excitation is not necessarily accompanied by an electrical change, and comes to the conclusion (p. 507) that none is free from

objection. We are, at present, entitled to hold the view that one is an aspect of the other.

Muscle.—This also has been already discussed (page 539). Fig. 205 is from a photograph by Keith Lucas of the diphasic response in the sartorius muscle, and Fig. 206 is one by Einthoven, with the string galvanometer. We have shown how it is to be explained on the basis of the theory of the origin of potential difference given in previous pages of the present chapter.

In muscle, just as in nerve, it appears that the electrical change is connected with the process of excitation; the excitation process is possible in muscle without the contraction process (page 539). The latter is a result of the excitation process, but may be unable to follow it. Fig. 172 (page 539) (middle curve) shows that, in the absence of calcium, the electrical change in the heart muscle takes place without any mechanical change. The significance of this fact has been pointed out above (page 398).

As to the precise location of the membranes concerned, both in nerve and in muscle, we must not forget the possibility that such polarised surfaces may occur, not only at the cell membrane on the outside of the cell, but at phase boundaries within it. But much more knowledge is required of the cell mechanisms.

The way in which the "demarcation" current, or current of injury, is to be explained has been described previously. A further word of explanation may be added here. If an uninjured cell, at rest, is led off from any two points on its outer surface, it is clear that they will be equipotential, since we are only dealing with the outer component of the double layer. If we could place one electrode *inside* the cell, we should obtain the potential difference between the two components of the layer, as in my experiments with Congo-red (1911, 2). This is, in fact, what we do when we cut through or injure a cell in contact with normal cells, leading off from the outer surface of the normal cells and from the injured cells. This latter contact is, in effect, the same as the interior of the cell. We saw above (page 393) how the disappearance of this potential difference on stimulation ("negative variation") is explained by the disappearance of the state of polarisation in the normal cells, owing to the membrane becoming

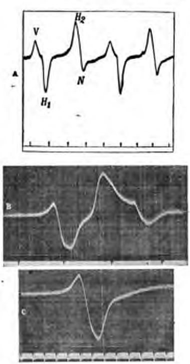


FIG. 207. ELECTRICAL CHANGES ACCOMPANYING THE PASSAGE OF A WAVE OF CONTRACTION ALONG THE URETER.—To be read from left to right.

A, Print from photograph given by string galvanometer.
Two complete waves.
Distance between electrodes—50 mm.
V, Electrode at which the wave first arrives becomes positive.
H_1, The chief negative wave.
H_2, Negativity of distant electrode.
N, Positivity of distant electrode.

B, Shows appearance of original photograph. One complete wave only is seen. The above four components are obvious. Time in seconds.

C, A wave which disappeared before reaching the second electrode, so that the electrical change under the first electrode only is shown, namely, the components *V* and H_1. Time in fifths of seconds.

(Orbeli and Brücke.)

Note that the figures are reproduced as sent me by Dr Orbeli. Those in the published paper are incorrectly given.

permeable to both ions of salts within the cell. It will be clear that the demarcation current can only last as long as the contents of the injured cell remain in place, and more or less identical in amount with what they were normally. As soon as the electrolytes have been replaced by those of the electrodes, owing to diffusion, the injured cells naturally become mere extensions of the leading off electrodes, and, being in contact with the normal surface of uninjured cells, the potential difference disappears.

It is interesting to note that in tissues which contain as much as 99 per cent. of water, such as those of the fresh water *Medusa*, investigated by Cremer (1906), an electrical change in contraction can be detected.

As an illustration of the phenomena in *smooth muscle*, we may take the *ureter*, in which the electrical changes accompanying a wave of contraction were recorded by Orbeli and Brücke (1910). Fig. 207 gives three of their curves. In curve B one wave only is given, and is a copy of the actual photograph as obtained. In curve A there are two waves, one of which is marked for description. A movement downwards means that the electrode under which the wave first passes becomes negative, and we notice that the large wave H_1 is in this direction, and that it is followed by another, H_2, in the opposite direction, as the wave of contraction leaves the first electrode and arrives at the second. This corresponds exactly to what happens in nerve and skeletal muscle. But what are the waves marked V and N? It is suggested by Orbeli and Brücke that they represent a wave of inhibition preceding the contraction, as we have seen in the case of the intestine (page 367). It is clear that, if the ureter were in a state of tonus or partial contraction, inhibition would cause the first electrode to become less negative than the second, and appear as a positive deflection. If, however, the deflection N is also due to progress of the wave of inhibition to the second electrode, as would appear from its opposite direction, it must have travelled at a slower rate than the excitation

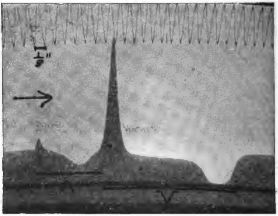


FIG. 208. ELECTRO-CARDIOGRAM OF TORTOISE.—Heart *in situ*. Led off from sinus and ventricle apex to capillary electrometer. Movement of shadow upwards means negativity of sinus contact.

A, Diphasic auricular response.
V, Diphasic ventricular response.
Temperature, 12° C.
Time in ⅕th sec.

(Gotch, 1910, p. 237.)

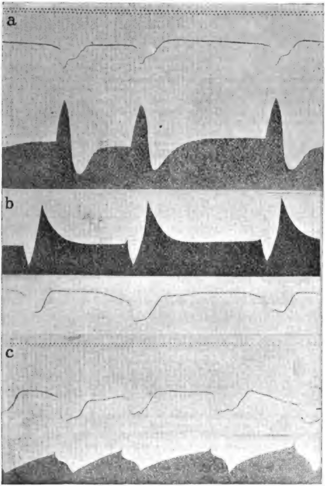


Fig. 209.

a, **Electrical change of the dog's ventricle *in situ*. Led off by contacts on apex and base.**
Warm air for artificial respiration.
Simple diphasic response, base becoming negative first. Time, $\frac{1}{5}$th sec. above.

b, **Reversed response with cold air for respiration.**
In middle curve, a trace of a preliminary short base negativity.

c, **An intermediate stage between *a* and *b*.**

The heart beats are indicated by a tambour giving the tracing between the time signal, and the electrical change, and are not to be taken as showing anything but the fact of the occurrence of a beat.

Note that although the electrical changes were opposite in sign in *a* and *b*, no difference was to be noticed in the nature of the beat, so far as could be judged by the eye.

(Bayliss and Starling, 1892, 1. Figs. 7, 6, and 5.)

wave, which seems improbable. On the other hand, the fact that, as shown in curve C, this fourth phase, as well as the third, is absent when the wave disappears before it reaches the second electrode, indicates that it is due to a disturbance, excitatory or inhibitory, which travels along the ureter. If it were an inhibition controlled by nerve centres, as in the intestine, it might perhaps have a different time course from the wave of contraction, but it would seem useless for it to follow the contraction wave at the further electrode. The inhibition wave sometimes occurs independently of the excitation, so that a series of monophasic responses in the positive direction may be seen, only occasionally followed by a negative one. The monophasic nature is due to the disappearance of the wave in a region of decrement before it reaches the second electrode.

In the spontaneous peristaltic waves in the *crop of Aplysia*, it was shown by Dittler (1911) that each wave consists of a simple negative change, without the electropositive accompaniment of that of the ureter. This wave is very slow, the total duration being about fourteen seconds, but it gives no indication of being anything but a single contraction, not a short tetanus. So far as could be made out, the duration of the mechanical response appeared to coincide with that of the electrical one; so that, if the latter is an expression of the excitatory state, and not of the state of contraction, the excitatory state in this case, at all events, must not merely precede that of contraction but last as long as the latter does.

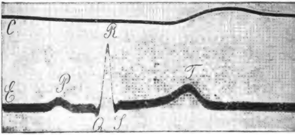


FIG. 210. TYPICAL FORM OF THE HUMAN ELECTRO-CARDIOGRAM, AS OBTAINED BY LEADING OFF FROM RIGHT ARM AND LEFT ARM (LEAD 2).

C, Carotid pulse.
E, Electro-cardiogram, with the designation of the component parts or waves as given by Einthoven.

Scale divisions of abscissæ, 0·01 sec.
Scale divisions of ordinates, 10^{-4} volts.

Einthoven regards *P* as the only component belonging to the auricle, *Q*, *R*, *S*, *T* all being parts of the ventricular complex (see text, page 657).

(Einthoven, 1913, p. 68.)

The Heart.—Apart from the interest of the phenomenon itself, the electrical change in the heart has become of great importance, not only as a means of following the course of the contraction, but as a clinical method of investigating irregularities in the heart beat (see the book by Lewis, 1913).

An exact analysis of the electrical change was first made by Burdon Sanderson and Page (1880), who showed that, in the frog's ventricle, a diphasic deflection occurred, similar to that which we have described in nerve and muscle. This was of such a direction as to show that the excitation process started at the base, and progressed as a wave to the apex. Fig. 208 is a photograph by Gotch (1910) of the phenomenon in the tortoise. In this case one electrode was on the sinus, the other on the apex of the ventricle, so that the auricular change is also shown, and is seen to be similar to that of the ventricle, but smaller. I made, in conjunction with Starling (1892, 1), observations on the corresponding change on the

mammalian heart. When electrodes were placed on the base and apex of the ventricle, in as normal a condition as we could maintain it, we found that the electrical change was of a simple diphasic character, indicating that the wave started at the base and was propagated to the apex, as shown in Fig. 209 (curve *a*). When, however, the phenomenon was investigated without opening the chest, as can be done by leading off from the right arm and left leg, there was found to be a third phase present, in the same direction as the first. The explanation which we gave was that the excitation process lasts longer at the base than at the apex, so that the electrical negativity at the apex has disappeared before that at the base has completed its time course. The subject was taken up by Einthoven, who invented the string galvanometer (1901, 1903, and 1904) for the purpose of the work. Fig. 210 shows the human "electro-cardiogram," with the designations of the components used by Einthoven (1913). It has been clearly shown that the first wave, P, is due to the auricular contraction, the second phase of the auricular contraction is usually concealed by the commencement of the first ventricular phase, R. The meaning of the small change, Q, is rather doubtful; it is not always present, and appears to belong to the ventricular "complex," as it is called. If so, it may mean that the ventricular excitation wave starts from a point a little distant from the base, or it may be merely a branch current, as it is so minute. The ventricular complex consists of the three deflections, R, S, and T. The first two obviously represent the commencement of the wave at, or near, the base and its progress to, and arrival at, the apex. But why is it cut short so quickly and followed by the third phase, which indicates excitation at the base? It is clear that the equipotential interval between S and T must mean that the whole ventricle is in a state of excitation; in fact, it corresponds to that part of the mechanical curve of the heart beat in which the entire ventricle is in a state of contraction. In further analysis, there are some facts to which Mines (1913, 3) calls attention, in a paper which contains an admirable account of the electro-cardiogram. There is no reason to suppose that the *t* wave is of any different nature from that of the other parts of the complex. It is the end of the total change, as shown by comparison with the monophasic change obtained when the one electrode is on an injured spot. As mentioned above, Bayliss and Starling (1892, 1) pointed out that it must be due to the electrical change at the base lasting longer than at the apex. It is in the same direction as the initial effect, R. This conclusion is confirmed by Mines (1913, 3, p. 201) in experiments in which, by warming the apex of the frog's ventricle, when it was giving a diphasic change, he caused the effect at the latter to take on a more rapid time course, and thus made the base negativity to last relatively longer. Fig. 211 shows that a curve similar to that of the human electro-cardiogram is obtained. But why, in the normal heart, should the base, which is excited first, apparently remain excited last? Gotch (1910) thought that it was due to the wave leaving the base, passing to the apex, and then back again to the base at the origin of the aorta. He brought it into relation with the development of the heart from a folded tube in the embryo. Meek and Eyster (1912) and Mines (1913, 3) have shown, however,

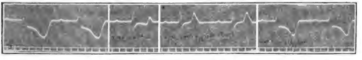


FIG. 211. EFFECT OF LOCAL WARMING ON THE ELECTRICAL CHANGE IN THE FROG'S VENTRICLE.

The first and last parts of the tracing show the normal diphasic effect before and after warming the apex. The second and third parts show the effect of shortening the duration of the excitatory process at the apex by raising the temperature. The relatively greater duration of the process at the base causes the appearance of the third phase, corresponding to Einthoven's T wave. The way in which this happens is shown in Fig. 212.

(Mines, 1913, 3, p. 200.)

that such an explanation does not hold. Details may be found in the work of Mines, who followed the course of the excitation wave by leading off from various points on the surface of the ventricle in the frog. No evidence was obtained of such a course of the wave as that supposed by Gotch. The only satisfactory explanation was found to be that the negativity at the base does actually last longer than that at the apex, when the heart is in position in the intact animal. Meek and Eyster come to the same conclusion. The way in which this fact accounts for the form of the electro-cardiogram will be clear from Fig. 212.

Now we must remember that, in the mammalian heart, the auricular excitation is transmitted to the ventricle through a system of special muscular fibres, Purkinje's cells, which branch to all parts of the ventricle, and that the muscular structure of the contractile wall consists of strands passing in various directions. It seems that Einthoven is inclined to attribute the form of the electro-cardiogram to excitation starting from a place not exactly at the base, but reaching the base before the apex, although various other parts, not necessarily the apex, might be excited immediately after the base. However the excitation wave is conveyed, it seems that at any given spot all the muscular layers must be in contraction simultaneously, otherwise there would be danger of their tearing apart. Moreover, the fact that simple hearts, such as those of the frog and tortoise, show, in the intact animal, similar forms of electro-cardiogram to that of the mammal, indicates that the development of the Purkinje system does not alter the general course of the wave. Electro-cardiograms of some of the lower vertebrates are given in Fig. 213 (from Lewis's book). It is probable, as Mines points out (1913, 3, p. 208), that the state of excitation lasts so much longer than the time taken for its transmission from one part to another, that a very small difference in the duration at one point or another is sufficient to determine the sign of the final phase. In fact, he noticed an alteration of sign in the final phase in a tortoise heart without any obvious difference in the beat.

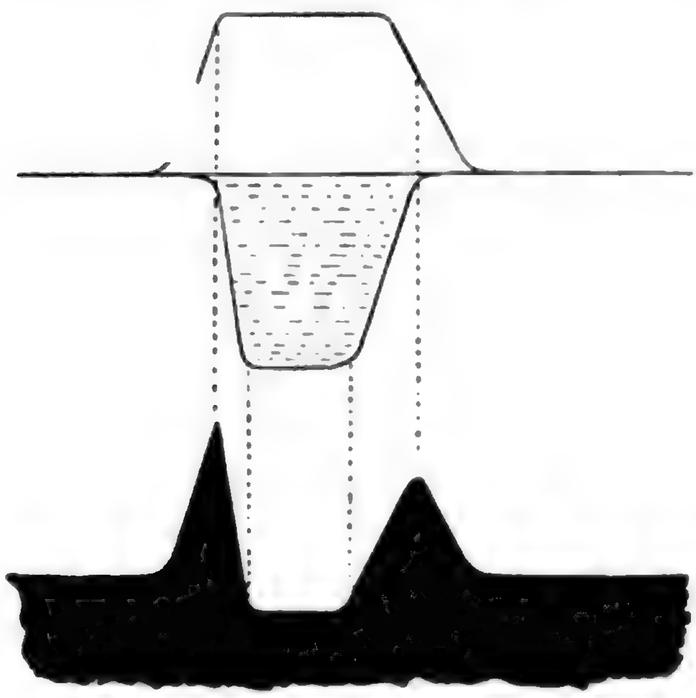


FIG. 212. DIAGRAM TO SHOW MANNER OF PRODUCTION OF NORMAL ELECTRO-CARDIOGRAM BY LONGER DURATION, WITH LESS HEIGHT, OF EXCITATORY STATE AT THE BASE THAN AT THE APEX.

Such a condition is brought about by warming the apex, for example.

The uppermost curve represents the excitatory state (negativity) at the base.

The middle one, that at the apex; represented in the opposite direction, since the ventricle is supposed to be led off by electrodes at base and apex.

The lowest curve represents the electrical change which would be seen with the capillary electrometer.

A detailed analysis of the course of the excitation wave in the dog and in the toad is given in the papers by Thos. Lewis (1915, 1 and 2).

From what has been stated, it will be clear that the chief practical value of the electro-cardiogram is in the detection of abnormalities in transmission from auricle to ventricle. Especially is it to be noted that conclusions based on changes in form of the ventricular complex rest on an uncertain basis until we know more about the precise meaning of its components. The very smallest difference between the time at which the excitation wave reaches two points decides which of these becomes negative first, although, as regards the mechanism of the contraction, the fact may be of no significance (see especially Figs. 209 and 211). There is, it may be repeated, no evidence that any component of the electro-cardiogram is due to a process different from any other component.

The whole is to be explained by difference in time relations of the ordinary wave of excitation in different directions. Caution must be exercised in drawing conclusions from the signs of the components. The electrical change, as recorded, does not merely indicate the magnitude of the process under one electrode only, since electrical expression of it is cut short according to the time at which the wave arrives at the other electrode.

The relations in time of the mechanical and electrical effects are of some

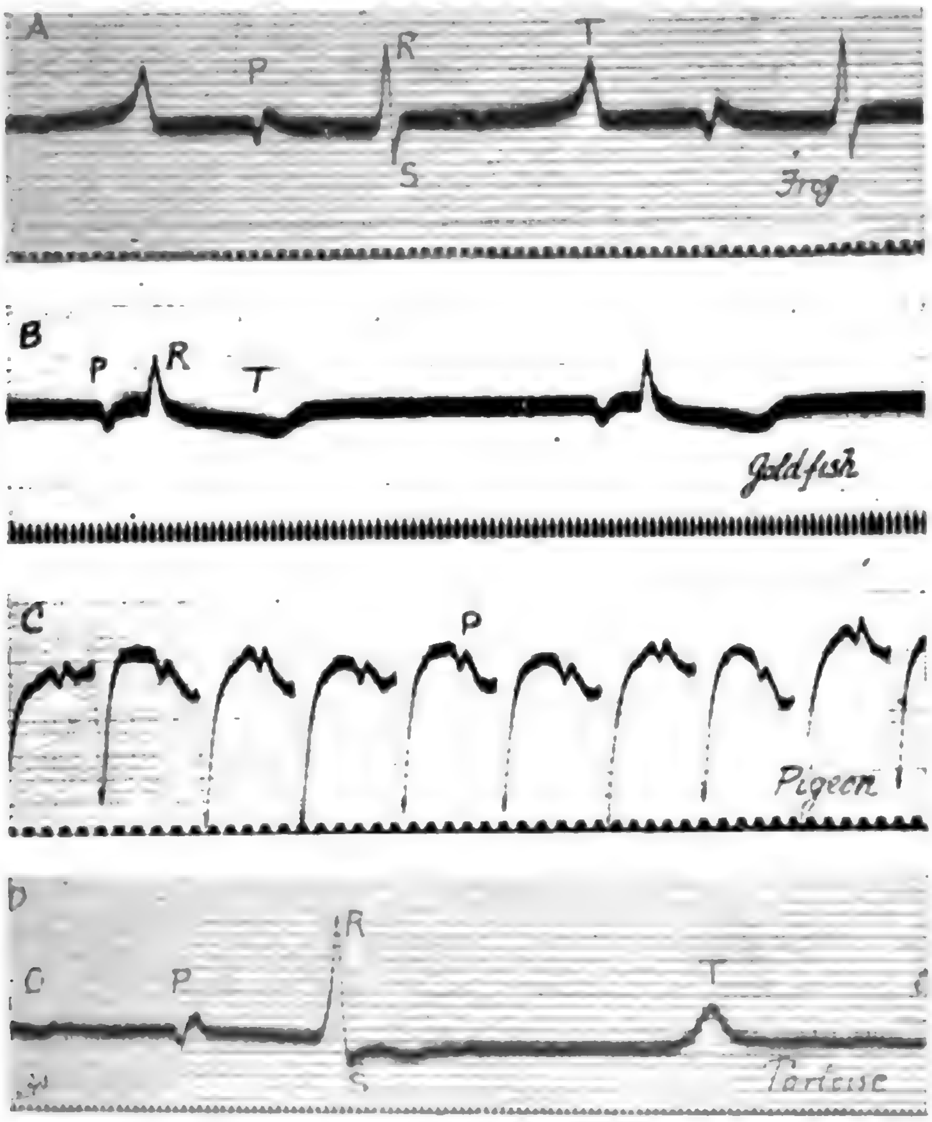


FIG. 213. ELECTRO-CARDIOGRAMS FROM DIFFERENT ANIMALS, TAKEN BY LEADING OFF FROM THE LIMBS WITH THE HEART UNEXPOSED.

Frog, goldfish, pigeon, and tortoise in order from above downwards.
The essential similarity to the human electro-cardiogram will be noticed in the cases of the frog and the tortoise. The goldfish shows a diphasic ventricular change, like the exposed heart of the frog and tortoise. That of the pigeon is peculiar.

(Lewis, 1913, 1, p. 18.)

interest. Figs. 172, 173, and 214 show that the duration is practically identical. In Fig. 214, from a short article by Piper (1913, 1), we see that the latter begins a little before the pressure change, and ends a little earlier. An interesting point is that the greater part of the final electrical phase appears to take place after the pressure curve has begun to fall, a fact which tends to confirm the view taken above that it represents the last part of the wave of excitation, namely, that part in the fibres which are the latest to relax.

It has been remarked above (page 215) that Lovatt Evans (1912, 2) found that the heart of the snail, although requiring calcium for its normal activity, is unusually insensitive to these ions. Thus, with 1 per cent. calcium chloride the beat is normal, while the frog's heart is sent into systolic contraction by this concentration. A peculiar effect on the electro-cardiogram is also to be seen. In Fig. 215 we see the effect referred to The heart is first in tonus; calcium

chloride, 0·6 per cent., is then applied, and subsequently a regular series of beats with a large initial deflection made its appearance. This disappeared again when the calcium salt was washed away with sodium chloride.

The electro-positive change occurring in inhibition, observed by Gaskell and others (p. 407), has been already discussed.

Secreting Glands.—The electrical changes in the salivary glands have been described above (pages 350-352). Fig. 93 (page 351) represents them. Certain conclusions as to the secretory process were drawn from them. Electrical effects in other glands were also mentioned, especially those of Hermann and Luchsinger on the frog's tongue and on the sweat glands of the cat (1878, 1 and 2).

The skin of the frog is also a structure containing simple glands, on which considerable work has been done. That of L. and E. Orbeli (1910), which contains full references to the earlier work, may be especially referred to here. These observers show that the direction of the response to nerve stimulation varies with the solution used on the leading off electrodes. With water alone the current is an inflowing one, that is, the outer surface becomes negative; with sodium chloride, 0·055 to 0·7 per cent., it becomes positive. With potassium chloride the effect is the same as with water, but preceded by a small deflection in the opposite direction. The interpretation of the facts is not easy, but the occurrence of an electrical change in the presence of water electrodes shows that it is not merely due to the ions of the electrodes. Also, the occurrence of two changes in two different directions indicates the existence of two processes in the gland cells, as discussed above (page 352).

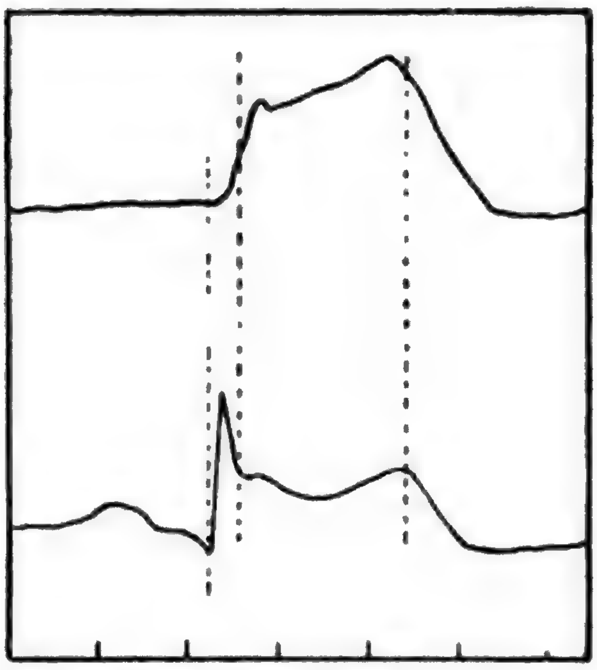


FIG. 214. SIMULTANEOUS RECORDS OF INTRAVENTRICULAR PRESSURE (UPPER CURVE) AND ELECTRICAL CHANGE (LOWER CURVE) OF THE CAT'S HEART.

Electrodes on auricle and ventricle. Time in tenths of a second.

Note that the electrical change continues during the period of relaxation of the ventricle.

(After Piper.)

If we suppose that we lead off from opposite ends of a gland cell and that one end becomes permeable when secretion occurs, it is clear that we obtain then the potential of the Helmholtz double layer, since we obtain access, as it were, to the interior of the cell. Thus, if the cell membrane is, at rest, permeable to certain anions only, we obtain an effect of the sign of that associated with stimulation of the chorda tympani nerve in the dog. This view is in agreement with the theory of secretion given above (page 334).

Electrical Fish.—The capability of certain fishes to give powerful electric shocks, amounting to a potential difference of two or three hundred volts, might appear puzzling until we remember that the electric organs are composed of a large number of plates, arranged in series, and that these plates are excited simultaneously by nerve fibres, so that a certain small potential difference is established between the opposite sides of each plate.

We see that there is no *wave* of excitation and, experimentally, the electrical change is found to be a discharge, or series of rhythmic discharges, in one direction only.

With the exception of that of Malapterurus, the electrical organs appear to be formed of modified skeletal muscle. It has been suggested by Gotch that the electrical change is that of the nerve end-plate. The muscular structure itself has almost disappeared, but Fig. 216 shows that an apparently complex arrangement of papillæ has taken its place. It is interesting to note that, although the organ of Malapterurus is developed from skin glands, its structure is very similar to that of other fish, so that there must be some significance in those parts present in addition to the nerve end-plates of the original muscle fibres.

The electromotive force of the shock, owing to the manner of connection of the plates in series, is naturally greater when these plates are arranged along the length of the fish than when across it. That of Malapterurus, according to the most recent determinations of Cremer, with the string galvanometer (see Garten's article, 1910, p. 200), amounts to 450 volts.

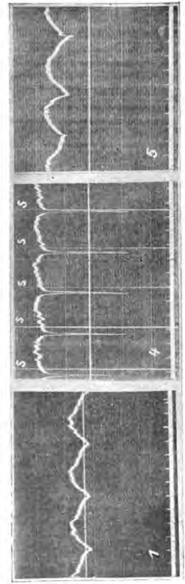


FIG. 215. EFFECT OF CALCIUM ON THE ELECTRICAL CHANGE OF THE SNAIL'S HEART.

1, Heart apparently in tonus, no visible beats.
4, After moistening with 0·6 per cent. calcium chloride. Beats commenced, but it is uncertain whether they were actually excited by the calcium. The sharp, rapid initial wave in the electrogram is due to the action of calcium, since it was abolished by washing with sodium chloride solution in curve 5.

(Evans, 1912, 2.)

It is obvious that, in order to obtain an effective potential difference, the whole of the electrical plates must be excited simultaneously. When they are all innervated from a single neurone on each side, as in Malapterurus (see page 471 above), this is easily explained. But in other fish the centres consist of a large number of cells, 106,000 in Torpedo, according to Fritsch. Since each single discharge of the organ only lasts about 0·005 sec., the adjustment of the reflex activity of the neurones must be very accurate in order that each plate shall be in phase with the others, so as to sum up with them.

The latent period appears to be no less when the organ is stimulated directly than when through the nerve. This fact may mean that the only active part is the nerve end-plate, or that any other part, such as might be developed out of the muscle fibre, is not directly excitable. After section of the nerves, the direct and indirect excitability of the organ vanish together, contrary to the case of muscle.

That there is something more than nerve fibre responsible for the electrical change is shown by the fact that the organ is much more quickly fatigued than nerve fibre itself, although not so quickly as muscle (Garten, 1910,

p. 184). The fatigue is simultaneous for direct and indirect stimulation, and is thus situated in the active cells themselves, not in an intermediate substance.

Experiments on heat production have shown that it is very minute; but the results are somewhat contradictory, and it has been suggested that two processes may be going on, an endothermic and an exothermic one. Correspondingly, only infinitesimal chemical changes have been detected.

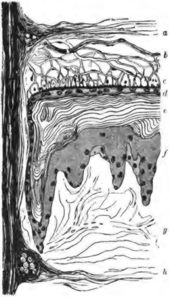


FIG. 216. ELECTRICAL ORGAN OF THE SKATE.—Microscopic structure of part of one of the discs. Somewhat diagrammatic.

a, Transverse septum
b, Medullated fibres of plexus } Nervous mina.
c, Terminal ramifications of non-medullated nerve fibres

d, Nucleated layer
e, Striated layer } The disc.
f, Alveolated

g, Connective tissue.

h, Transverse septum. At its junction with the longitudinal septum a nerve is seen in section.

(Burdon-Sanderson and Gotch, *Jl. of Physiol.*, 9, p. 143.)

The conclusion seems to be indicated that the process is one involving very slight expenditure of energy, apart from that necessary for the current itself. Probably the chief process is one of change of permeability, involving redistribution of ions.

The electric fish are relatively insensitive to electrical shocks, although not entirely so, as was thought at one time. This state may, perhaps, depend on some peculiarity of the permeability of their cell membranes to ions; that is, it may be a bad electrolytic conductor, as suggested by Garten (1910, p. 212).

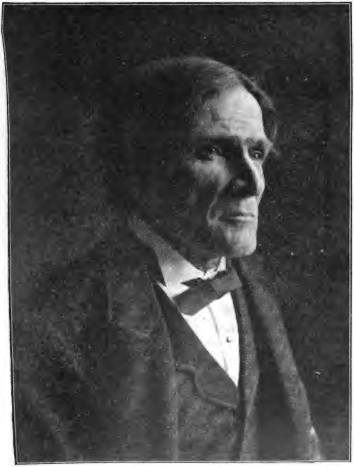
The Retina.—The significance of the electrical change in this organ has been discussed above (pages 523-524).

Plant Tissues.—In accordance with the general theory of the mode of production of differences of electrical potential, it would be expected that all phenomena associated with changes in the permeability of the cell membrane would give rise to electrical effects. Owing to the short circuits present in all tissues, it is only when the processes take place with some rapidity that it is possible to detect them.

Some cases have already been given. Dionæa and the sensitive plant (page 430), the effect of light on the green leaf (page 567), and the moving protoplasm of Nitella may be mentioned.

Loeb and Beutner (1912) and Beutner (1912) made some interesting experiments on the apple. If both electrodes are on uninjured surfaces, change of the electrolyte concentration in one of the electrodes causes the appearance of a potential difference, owing to change of concentration of the ions forming the double layer, and the magnitude of the effect follows the Nernst formula as given above (page 191). The addition of non-electrolytes, urea or sugar, does not affect the potential difference. All cations act in the same way, as was explained above (page 161), owing to the possibility of free interchange between ions of the same sign. It may be remarked that this interchangeability as regards all cations distinguishes the kind of concentration battery in question from that where metallic electrodes are concerned. In the latter case, of course, it applies only to salts of the particular metallic ion itself. In the former case, the electrode, by interchange, becomes one composed of all the cations present outside it, in corresponding concentrations.

The reader will probably have noticed how much of the fundamental work in the region of the phenomena of electrical responses is due to Burdon-Sanderson and his co-workers. Skeletal muscle, heart, plant tissue, and the electric fish received the greater part of their clear and definite presentation from the researches mentioned. This is, therefore, the most appropriate place to call attention to his portrait, which will be found in Fig. 217.



J Burdon Sanderson

FIG. 217. PORTRAIT OF BURDON-SANDERSON.

(From a negative by Sir Ed. Schäfer.)

SUMMARY

In the use of instruments for recording the changes in the electrical state of tissues, the important point to be kept in mind is that the moving parts shall be able to follow rapid alterations in current or potential, and this without overshooting the correct position. They must either be aperiodic, but without more damping than just necessary, or their own vibration period must be shorter than that of any change to be measured.

The interest of electrical changes is not only as giving insight into the processes going on in the cells, but also as a means of investigation of the time relations and other properties of these processes. In the case of nerves, there is frequently no other method available for detecting the existence of the passage of impulses.

Although the ultimate source of differences of potential in cells must be due to the separation of electrically charged ions, it is found that the different rates of movement of ions free to diffuse is inadequate, while the presence of metallic electrodes similar to those of the usual form of concentration battery is out of the question.

On the other hand, the existence of a cell membrane permeable to one only of the oppositely charged ions of a binary electrolyte is capable of accounting satisfactorily for all the phenomena met with.

It is shown that the electromotive force of a concentration battery of this particular kind follows the same formula as that with metallic electrodes. It may, indeed, be regarded as a model of the process in the case of metallic electrodes. The difference is that the membrane cell, or electrode, is indifferent to the chemical nature of the ions, being concerned only with the *sign* of the charge, while the metallic electrode only takes account of ions of the same chemical nature as itself. The reason for this difference in behaviour is that the membrane allows free interchange between diffusible ions of the same sign between the interior and exterior, so long as the potential difference is unchanged thereby.

The contact surface between phases is, similarly, the site of a potential difference, if one of the ions is soluble in both phases, the other in one only.

There is reason to believe that the electrical change in nerve and muscle is inseparable from the state of excitation, but that the state of contraction of muscle may be absent, although the electrical phenomena remain.

Description is given in the text of the way in which the "demarcation current" and the "negative variation" in nerve and muscle are explained on the basis of membrane potential.

The phenomena in smooth muscle are discussed in the text.

In the ventricle of the heart, when led off directly, there is a simple diphasic change, indicating the progression of a wave of negativity from base to apex.

When the electro-cardiogram is obtained from the unexposed heart it has three phases, the third one indicating negativity of the base. This may be due either to the excitatory state lasting longer at the base than at the apex, or be due to the course of the wave not being so simple as a progression from base to apex merely. The former hypothesis is more in accordance with facts. But it must be remembered that transmission is by means of Purkinje tissue, which conducts faster than ordinary heart muscle, so that contraction may be practically simultaneous in all parts of the ventricle. By shortening, artificially, the duration of the excitatory state at the apex, or lengthening that at the base, the triphasic curve can be obtained from a diphasic one.

There is no reason to suppose that there is any difference between the components of the electro-cardiogram other than that due to the different times of arrival and duration of the excitatory state at the various parts of the heart muscle.

In the skin of the frog, it has been shown that the electrical change can still be obtained when the saline solution in the leading off electrodes is replaced by water; so that a clear proof is given that the electrical effect is not merely due to the electrolytes of the leading off solution. In accordance with the membrane theory, we find that both here and in the case of plant tissues the electromotive force is modified by the concentration of the cations of the leading off solution.

A brief account is given in the text of the electrical fish, and the interest of the phenomena from the general point of view, as regards reflex có-ordination and so on, is pointed out.

The electrical phenomena in plants are readily explicable in terms of changes of permeability of the cell membrane.

LITERATURE

Theory.
Bernstein (1902 and 1913). Beutner (1912). Bayliss (1911, 2).

General Facts.
Garten (1910).

Voluntary Muscle.
Keith Lucas (1908, 2, and 1909, 2). Piper (1912, 1).

Electro-cardiogram.
Mines (1913, 3). Einthoven (1913). Lewis (1913, 1).

Methods.
String Galvanometer. Einthoven (1901, 1903, and 1904).
Capillary Electrometer. Burch (1890 and 1892). Keith Lucas (1909, 2).

CHAPTER XXIII

THE CIRCULATION OF THE BLOOD

In organisms of microscopic dimensions there is obviously sufficient opportunity for interchange of soluble materials by diffusion, without the necessity for special provision to enable the products of the activity of one organ to reach another organ with due rapidity. With increase in size this is no longer the case, and is especially important with regard to the supply of oxygen.

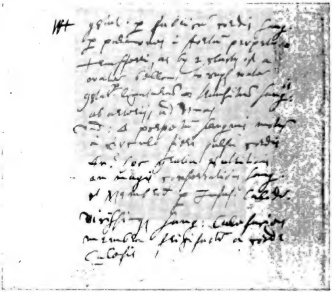


Fig. 218. A page from the reproduction of Harvey's manuscript notes.

(Harvey, 1616, p. 80.)

Accordingly, we find more or less rudimentary methods of causing flow of body fluids from one part to another in early stages of evolution.

The objects of such currents, which later become developed into those of a special fluid, the blood, in a system of closed tubes with a pump, the heart, may be said to be, in general, threefold :—

1. The supply of food, inclusive of oxygen, to all parts of the organism.
2. The removal of waste products of activity, which would paralyse function if allowed to accumulate.
3. The carriage of chemical substances from an organ in which they are

produced in order to influence the activity of other organs. This function has come especially into prominence in recent years in the theory of internal secretions, or hormones.

FIG. 219. PORTRAIT OF HARVEY.

(Photographed by Elliott & Fry from the picture by Mierevelt in the Council Room of University College, London. The original is in the possession of University of London, University College. Reproduced by permission.)

In the early stages this fluid is not confined to special channels, but passes

through interspaces in the tissues. A kind of pump, the heart, is very early met with, even in such simple vascular systems, and it serves to accelerate the flow. In some organs in the higher vertebrates there are remains of this lacunar system of circulation, in the spleen, for example.

History.—It is a matter of common knowledge that Harvey (1616 and 1628) was the first to grasp the fact that the function of the heart is to drive the blood along the blood vessels and back to the heart in a circle. This knowledge he gained by experiments on living animals, combined with deductions from the direction in which the valves of the heart and of the veins allowed the blood to flow. The first statement appears to be in the manuscript notes of lectures given in 1615 to the College of Physicians of London. These notes have been published in facsimile by the College of Physicians, at the instance of the late Sir Edward Sieveking. The reader may be interested to glance at Fig. 218, in which a page from this facsimile is reproduced. The statements to which Harvey affixes his initials, he intends to claim as his discoveries. The transcription of

FIG. 220. PORTRAIT OF LEEUWENHOEK.

(From the reproduction in *Jl. R. Micros. Soc.*, 1913, part 2. By the kindness of the Council.)

the first eight lines of this passage, as given in the book referred to, reads thus:—

> "WH constat per fabricam cordis sanguinem
> per pulmones in Aortam perpetuo
> transferri, as by two clacks of a
> water bellows to rayse water
> constat per ligaturam transitum sanguinis
> ab arteriis ad venas
> unde △ perpetuum sanguinis motum
> in circulo fieri pulsu cordis."

It is to be remembered that these notes were only meant to be used by the lecturer to assist his memory, so that a translation must be somewhat free. It may be given thus: "WH shows, by the way the heart is made, that the blood is perpetually driven from the lungs into the aorta, 'as by two clacks of a water bellows to rayse water.' He shows, by means of ligature, the passage of the blood from arteries to veins. Hence it is demonstrated that the perpetual movement of the blood takes place in a circle, owing to the beat of the heart."

The more complete demonstration was given in the book published in 1628. A portrait of Harvey is given in Fig. 219.

The demonstration of the actual passage of blood from arteries to veins in the peripheral parts of the circulatory system was first given by Leeuwenhoek in 1686 (see H. G. Plimmer, 1913, p. 130). He saw it in the tail of the tadpole by the aid of the microscope which he had invented. A portrait of Leeuwenhoek will be found in Fig. 220.

Malpighi in 1661 (see Foster, 1901, p. 97) saw the capillaries in the dried lung of the frog, but it was Leeuwenhoek who first detected, in the living animal, the blood actually passing from artery to vein through the capillaries.

Although the first clear presentation of the circulation of the blood was made by Harvey, it is plain that Leonardo da Vinci (1452-1519) was not far from the discovery. It seems evident from his descriptions in the "Quaderni d'Anatomia" that he realised that the function of the heart is to drive the blood into the arteries. One of his drawings of the heart and blood vessels in man is given in reduced size in Fig. 221, and some sketches illustrating observations made on the movements of the heart of the pig, when killed by inserting a "piercer" for wine casks into the heart, in Figs. 222 and 223. In the description of these observations he states, "il core nella sua espulsione del sangue si racorta," "the heart shortens itself during its expulsion of the blood" ("Quaderni d'Anatomia," I. p. 22. Line 8 of Fig. 223).

In Fig. 221 the curious "mirror" writing used by Leonardo will be noticed. It is sometimes stated that this is a proof that the artist was left-handed. This view is confirmed by the fact that his shading is always drawn from left to right downwards. Others hold that it is much more probable that he could use either hand equally well, and adopted the mirror writing as a protection against ecclesiastical interference, since the nature of the writing was not discovered for a considerable time, and it was thought to be a cipher.

Since the blood vessels, as they get further from the heart, divide up into smaller and smaller branches, it will be clear, from the account given on page 241 above, that the internal friction of the blood causes considerable resistance to the flow. A somewhat high pressure is thus required in the main arteries to drive the blood at an adequate rate through these small vessels. It may be repeated here that it is in the small arterioles that the chief resistance occurs, on account of the fact that the rate of flow is great here, and the friction is proportional to the velocity. In the capillaries, although they are, individually, narrower than the arterioles, the rate of flow is small, owing to the sectional area of the bed being greatly increased by the great increase in their number. A high arterial pressure is also of advantage when the arterioles of an active organ are dilated. The high blood pressure enables a considerably greater flow to take place through the organ, without notable diminution of that through other organs. Moreover, a much more

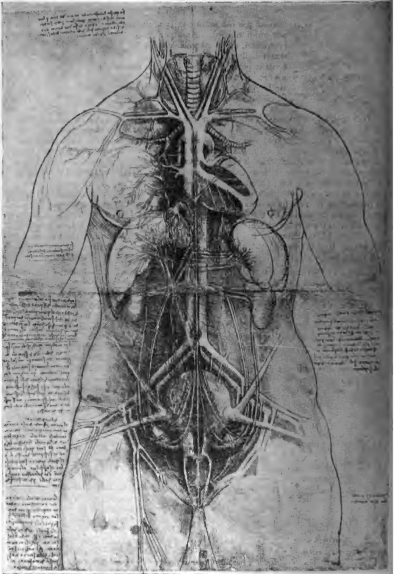


FIG. 221. COPY OF THE DRAWING BY LEONARDO DA VINCI OF THE ANATOMY OF THE HUMAN VASCULAR SYSTEM.—Reduced in size.

(Leonardo, 1452-1519, Folio, 12 recto.)

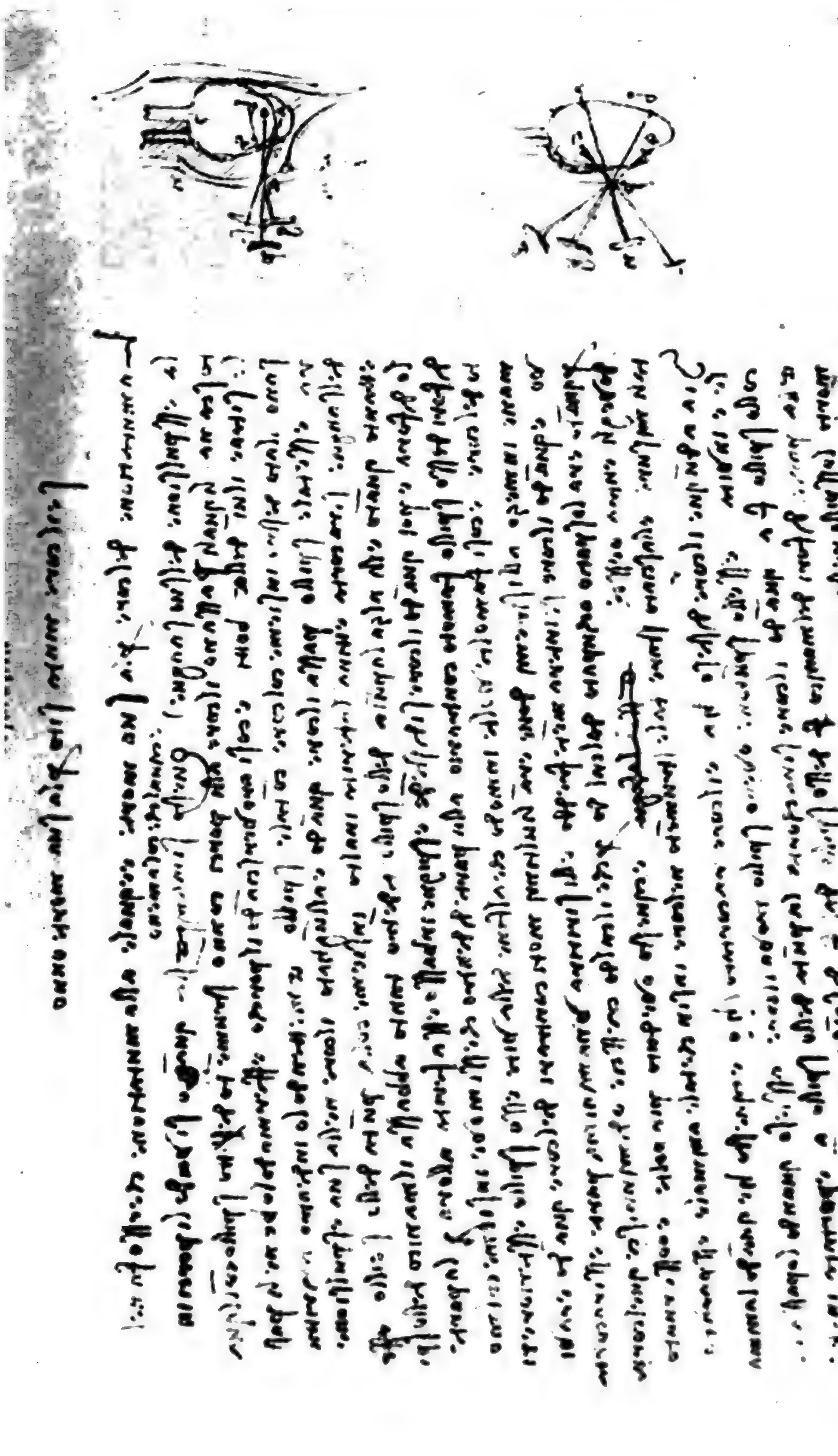


FIG. 222. LEONARDO'S DESCRIPTION AND DIAGRAMS OF HIS EXPERIMENT ON THE BEAT OF THE HEART.—It seemed better to reproduce the writing in the original size, although this necessitates the omission of part of the page. It will be found possible to read it with the aid of a mirror.

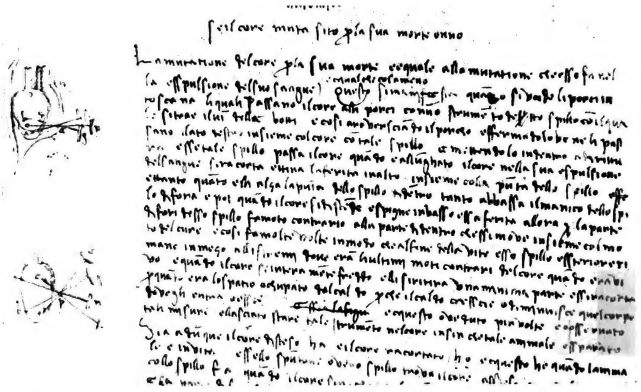


FIG. 223. A portion is shown enlarged and as seen in a mirror. It was actually obtained by enlarging the negative with the glass side outwards, a process which is equivalent to observation in a mirror. A translation of the description of the heart movements will be found on page 667 of the text.

delicate adjustment of flow can be effected with a high driving pressure than with a low one.

The fact that mammals possess a high arterial pressure was shown by Stephen Hales (1733). The second volume of his book, "Hæmastatics," opens with the description of his famous experiment, which I will give in his own words:—"In December I caused a *mare* to be tied down alive on her back . . . having laid open the left crural artery about 3 in. from her belly, I inserted into it a brass pipe whose bore was $\frac{1}{6}$ of an inch in diameter; and to that, by means of another brass pipe which was fitly adapted to it, I fixed a glass tube, of nearly the same diameter, which was 9 ft. in length: then untying the ligature on the artery, the blood rose in the tube 8 ft. 3 in. perpendicular above the level of the left ventricle of the heart: but it did not attain to its full height at once; it rushed up about half way in an instant, and afterwards gradually at each pulse 12, 8, 6, 4, 2, and sometimes 1 in.: when it was at its full height, it would rise at and after each pulse 2, 3, or 4 in.; and sometimes it would fall 12 or 14 in., and have there for a time the same vibrations up and down, at and after each pulse, as it had, when it was at its full height; to which it would rise again, after 40 or 50 pulses."

FIG. 224. PORTRAIT OF STEPHEN HALES.

A portrait of Stephen Hales is given in Fig. 224.

The circulatory system, then, consists of a branching system of tubes, the arteries, arising from a pump, the heart. After dividing up into a fine network of capillaries in the various organs, the vessels reunite to veins, which enter the heart again at the opposite end.

In mammals there are what amounts to two separate circulations with two pumps, although the two pumps are combined together side by side in the heart,

where they are called right and left auricles and ventricles, respectively. Fig. 225 is a schema of the circulation in birds and mammals. In the fish there is only one circulation. The venous blood, arriving at the heart is sent first through the aerating organs, the gills, from which it is distributed to the arteries

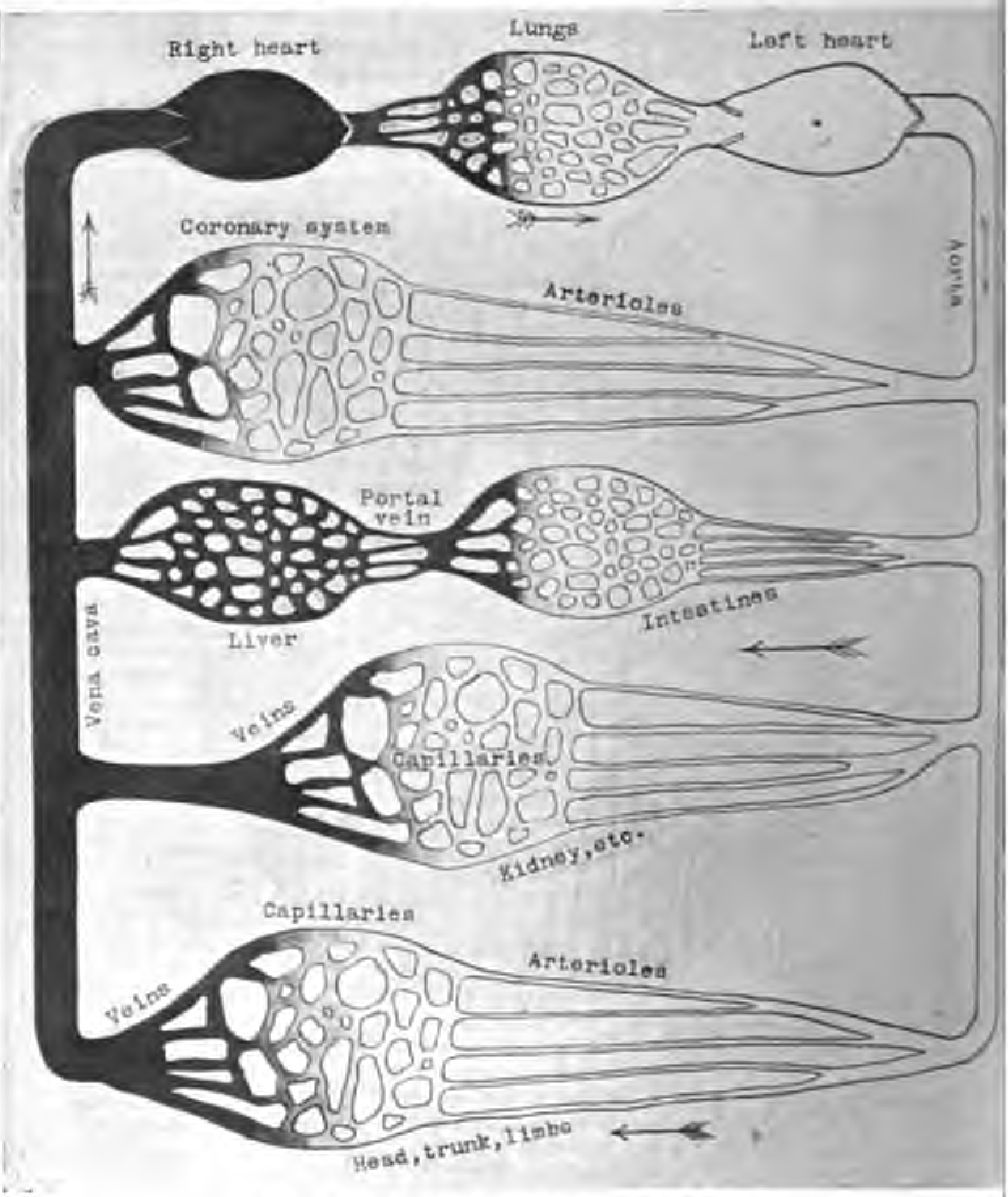


FIG. 225. DIAGRAM OF THE MAMMALIAN CIRCULATION.

Venous blood, black. Arterial blood, left white.

of the body in general. In the amphibia and reptiles, part of the blood only passes through the lungs, which may be considered to be one of the parallel paths of the diagram. There are, however, arrangements by which a more or less perfect separation of the aerated blood from the venous blood is effected, so that the organs may have the benefit of that which contains most oxygen. Details of

these arrangements may be found in the textbooks of comparative anatomy. In the schema, the blood which has lost its oxygen to a great extent, and taken up excretory products, is represented black. As is well known, this blood appears blue by reflected light through the skin. "Blue blood" is that which is of comparatively little use for the demands of the organism.

THE HEART

As remarked above, Leonardo da Vinci realised that the heart is an organ which, by its active muscular contraction, decreases periodically in its volume, and thus drives out the blood which has run into it during the time in which it was relaxed. Owing to the high pressure necessary to drive the blood through the peripheral arterioles, it is clear that, unless there were valves at the origin of the aorta to prevent the blood flowing back, this pressure could only last for a moment, during the contraction of the ventricle, and also that no blood, or very little, could run in from the veins, since the ventricle would fill up from the aorta. In fact, there would be a very inefficient circulation. Valves are obviously necessary between the auricles and ventricles also, to enable the energy of the ventricular contraction to drive the blood into the aorta or pulmonary artery against the pressure existing there, and not backwards to the veins.

Owing to its great importance, the physiology of the heart and the circulation has probably attracted more attention than any other branch of the science. It is clearly impossible to refer to the whole of this work, so that I must confine myself to facts which seem to be of the most general interest. Further details may be found in the book by Starling (1920).

The general properties of the muscle of the heart have been described on pages 451-454 above.

The Work of the Heart

The energy given out by the muscular contraction of the ventricles is, apart from the heat produced, used in raising the pressure in the aorta and in giving to the mass of blood a certain velocity. The former is mainly expended, to begin with, in stretching the elastic walls of the arteries. The kinetic energy of the latter is only a small fraction of the whole work when the output is small. According to the data given in Starling's book (1915, p. 916), in the human heart the kinetic energy only amounts to 0·7 gram-metre per beat; whereas the former, measured by the product of the volume of the blood driven out by the pressure to which it is raised, amounts to about 81·6 gram-metres. On the other hand, when the output is large, as in muscular work, the kinetic energy of the blood current is an appreciable fraction of the total external work of the ventricular contraction. Evans finds, for example, that with an output of two litres per minute, the kinetic energy amounts to as much as 16 to 18 per cent. of the whole. In the case of the right ventricle, owing to the low pressure in the pulmonary artery, the product of volume and pressure is probably a much smaller fraction of the whole work than in that of the left ventricle. The pressure here is taken as the mean between the aortic pressure at the moment of opening of the aortic valves and that when they close again as the ventricle begins to relax. It is obvious that, for an accurate estimation, we need to know the time course of the pressure and to determine the integral of it.

Intra-ventricular Pressure

In order to determine the time curve of the pressure change, a manometer is necessary which is capable of following exactly the changes of pressure without distortion by inertia of moving parts, and so on. The first approximation to such an instrument was made by Starling and myself (1894), and the curves we obtained were very similar to those which Piper (1912, 2, and 1913, 2 and 3) has published as the results of a much more perfect method. This method, also an optical one, is described in the first of the papers

named. Fig. 226 is a reproduction of a simultaneous tracing of intra-ventricular

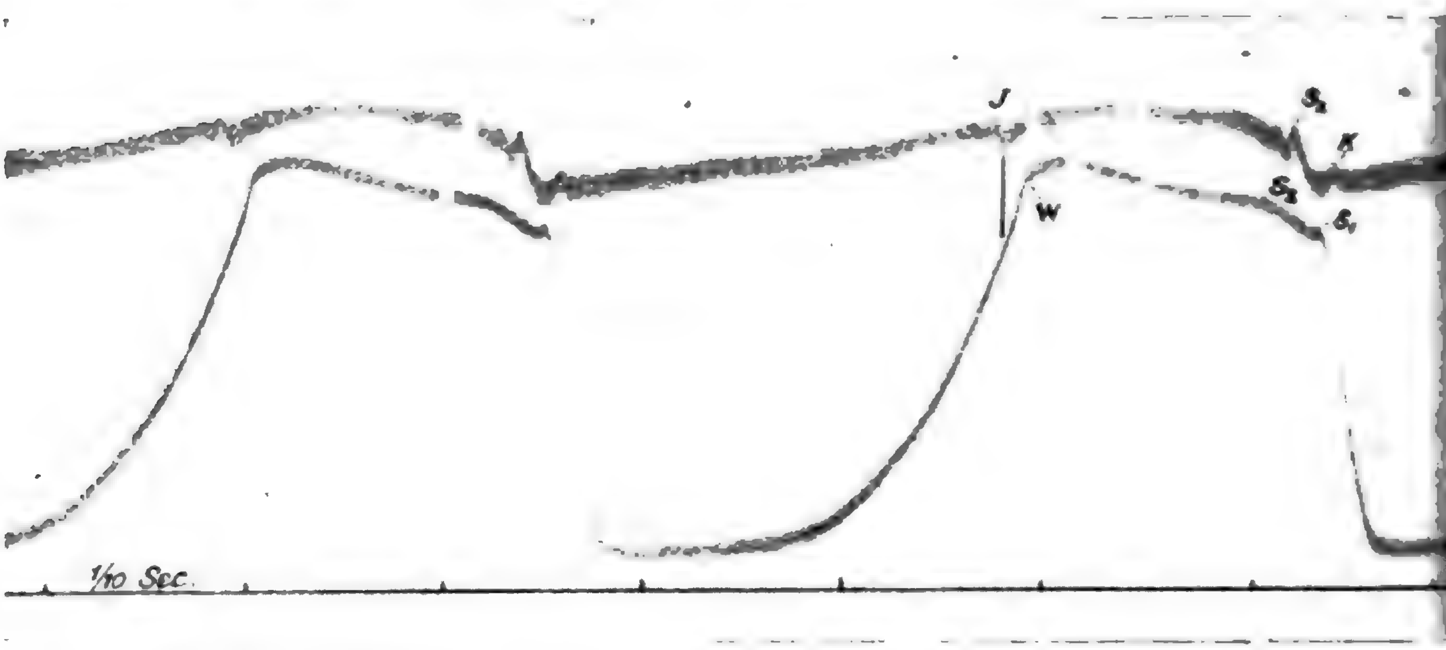


FIG. 226. PRESSURE CURVES FROM AORTA (UPPER TRACING) AND LEFT VENTRICLE (LOWER TRACING).—To be read from right to left.

Note that the aortic pressure does not begin to rise until the intra-ventricular pressure has risen to the level of S_1. The wave at S_1 on the ventricular curve lies in time between K and S_2 of the aortic curve. The notch J of the aortic curve is considerably later than the beginning (W) of the fall of pressure in the ventricle.

(Piper.)

and aortic pressures, and Fig. 227 a diagram showing the relations between

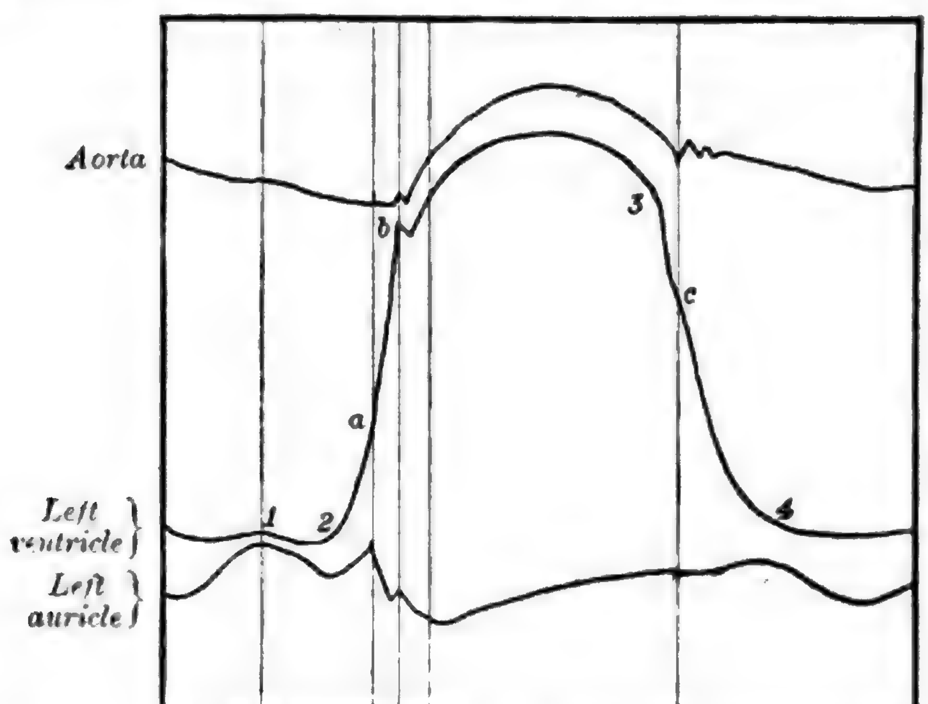


FIG. 227. CURVES SHOWING SIMULTANEOUS CHANGES OF PRESSURE IN THE AORTA, LEFT VENTRICLE, AND LEFT AURICLE, IN ORDER FROM ABOVE DOWN.—To be read from left to right.

1, Systole of auricle.
  a, Closure of auriculo-ventricular valve.
  b, Opening of aortic valve.
3, Sudden fall of intra-ventricular pressure.
  c, Closure of aortic valve.
4, Slow rise of pressure in auricle, due to inflow of blood from the veins. This pressure falls again as soon as the relaxed ventricle allows blood to enter it.

(After Piper.)

the pressures in left auricle, left ventricle, and aorta. The chief points to be noted

are that the auricular contraction (1) produces only a slight increase of pressure in the ventricle, since the latter is quite lax. As the ventricle contracts, there is a small rise of pressure in the auricle, due to pressing back of the mitral valve as it closes. The ventricular pressure rises rapidly, without any increase in aortic pressure, since the semilunar valves are closed by the greater pressure in the aorta. As soon as the intra-ventricular pressure is slightly greater than that of the aorta (at *b*), these valves open and the aortic pressure rises simultaneously with the further rise in the ventricular pressure, the two curves being practically parallel, until the ventricle commences to relax. Since blood is flowing from ventricle to aorta during this period, the pressure in the former must be somewhat higher than in the latter, and we notice that it is not until the ventricular pressure has fallen somewhat, at the line *c*, that the semilunar valves close, marked by a series of vibrations on the aortic curve. The further course of the ventricular pressure curve is independent of that of the aorta. The significance of the remaining points marked on the curve will be found in the description of the figures. The actual shape of the top of the ventricular pressure curve, during the time of driving blood into the aorta, varies according to the resistance in the arterial system. It may be dome-shaped or have indications of waves on it, but, on the whole, it is a kind of plateau, compared with the rapid rise and fall. It appears, then, that the form found by Chauveau and Marey, and confirmed by Bayliss and Starling, is the correct one, although the waves are somewhat exaggerated in these, especially in Chauveau and Marey's. The more or less sharp-peaked curves, obtained by some investigators, are due to insufficient accuracy of response of the instrumental method used.

There is no change in length of the muscle fibres of the ventricle until nearly the full height of contraction is reached. Now A. V. Hill (page 443 above) showed that the maximal external work is obtained from skeletal muscle if not allowed to shorten until the full state of tension is developed, so that the heart muscle works, in this respect, under nearly optimal conditions. Owing to the curvature of the heart, however, it is clear that only a part of the force of the contraction is exerted in the direction required, namely, inwards, so that the fibres act at a considerable mechanical disadvantage.

The researches of Patterson and Starling (1914) show that the work done by the heart is determined by the amount of blood flowing into the ventricles from the venous side. Up to a very high rate of inflow, the power of raising this volume to the aortic pressure is fully adequate. The limit at which this power fails is much higher than had been supposed from previous experimental work, in which sufficient venous supply was not provided. Put in another way, the energy produced in a ventricular contraction is in direct proportion to the *length* of the muscle fibres at the time when contraction begins. Thus the heart muscle obeys a similar law to that of skeletal muscle, in which we saw (page 443) that the energy developed is in relation to the magnitude of certain *surfaces* in the fibres.

Patterson, Piper, and Starling (1914) show in more detail how the length of the muscle fibres during contraction determines the output. The energy of each systole is proportional to the preceding diastolic volume. That it is not the initial tension that controls the force of contraction is shown by the experiments given on p. 502 of the paper. The heart of the tortoise obeys the same law (Kozawa, 1915), which is capable of explaining in a satisfactory manner all the facts relating to the output of the heart.

The function of the *auricle* is to collect sufficient blood, during the time the auriculo-ventricular valves are closed, to fill up the relaxed ventricle, when the auricle contracts (see Gesell, 1916). By storing up blood in this way, distension and rise of pressure in the veins is prevented.

The Heart Sounds

The heart sounds have for centuries attracted attention, chiefly owing to their use in clinical diagnosis. Thos. Lewis (1913, 2), by an improvement in the microphone method of Einthoven, has obtained interesting records with the string galvanometer, using two parallel strings, so that the electrical change of the muscle can be recorded at the same time. This addition to the instrument

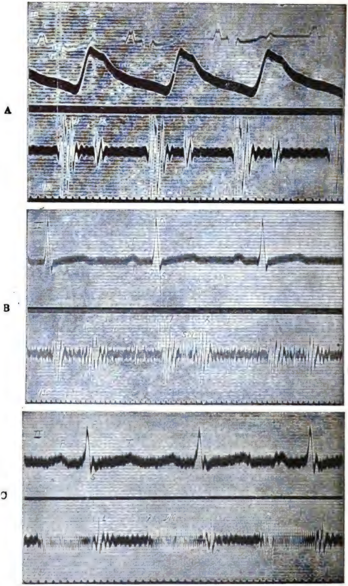


FIG. 228. RECORDS OF HEART SOUNDS.—Taken simultaneously with the carotid pulse and electro-cardiogram.

Uppermost curve, electro-cardiogram (Lead II.).
Lowest curve, movements of a second string of the galvanometer, connected with a microphone over the heart.
A, Normal record from the dog.
The carotid pulse is interposed between the other two. Time in $\frac{1}{50}$th sec.
The continuous vertical line marks the beginning of the ventricular systole, the dotted line the end.
B, Heart sounds from a patient with incompetent mitral valve. The murmur (systolic) is at *SM.*, of rapid rate of vibration, and in the middle of the ventricular systole.
C, From a case of incompetent aortic valves, giving a musical murmur during the whole of diastole.

(Lewis, 1913, 2, Figs. 3, 15, and 18, *Quart. Jl. of Med.*)

has shown itself very valuable also in comparing the electro-cardiograms from different leads. Fig. 228 gives three records, one a normal record from the dog, one from a human patient with a systolic mitral murmur, due to escape of blood through the imperfect valve during systole, and a third in which there was a musical murmur during diastole, due to incompetence of the aortic valves.

The second sound, a sharp one, is caused by the sudden tension put on the aortic valves as they are shut by the aortic pressure when the ventricle begins to relax. The first sound, of a softer and more prolonged character, appears to be due to two causes; one, the closure of the auriculo-ventricular valves, the other the muscular contraction, since it can be heard in the excised, empty heart. The pitch of this second element is the same as that of the resonance of the ear passage, which exaggerates the vibrations which correspond to its own period.

Relation of Activity to Oxygen Consumption

Some facts relating to this question have been already given (page 612). The work of Rohde (1912) and of Rohde and Nagasaki (1913) requires a little more detail.

In order to be able to control the conditions, the isolated perfused heart (cat or rabbit) was used in the method described in 1910, with the improvement of placing the whole in a thermostat. The object was to determine the relation between the activity and the chemical changes, including the consumption of oxygen and of glucose, the production of carbon dioxide and of other end products.

The first result is identical with that of A. V. Hill, already mentioned, on skeletal muscle, namely, that there is a direct proportionality between the oxygen consumption and the pressure developed in isometric contraction, in which the volume of the heart does not change. An important point is that, although the pulse rate is considerably lower at 15° than at 36°, the oxygen consumption per millimetre pressure developed is the same, within the limits of experimental error, namely, $427\text{-}436 \times 10^{-7}$ c.c. of oxygen. This fact of the absence of temperature effect on the conversion of chemical to mechanical energy is, no doubt, an important one from the point of view of energetics; what it means is not yet clear, although the surface energy as a "limiting factor" is indicated.

From the work of Zuntz and his co-workers on the whole animal we know that we can convert the oxygen consumed into its equivalent of oxidised food-stuff, in the proportion of calories developed. This was found to hold in the heart. The ratio of pressure developed to calories produced by oxidation was the same, whether glucose with a respiratory quotient of 0·98, or the "reserve stuff" of the heart itself, with a respiratory quotient of 0·80, was consumed.

In short, the ratio of the chemical energy of oxidation, or calories, to the amount of pressure developed is a constant number.

To obtain further insight into the mechanism of the energy change, experiments were made in which the heart was placed under abnormal conditions, narcotics, want of oxygen, the influence of muscarine, of veratrine, etc. It was found that the heart muscle worked less efficiently; that is, less pressure was developed in proportion to the oxygen consumed. As a first step towards the analysis of these results, the behaviour of the heart as regards different food-stuffs was investigated. When glucose was present in the circulating Ringer's solution, the carbohydrate was consumed along with certain reserve materials present in the cells themselves. As to the chemical nature of these "reserve stuffs" we have little information. There is no evidence that protein is consumed in muscular contraction. In fact, the experiments of Athanasiu and Gradinesco (1912) seem to show that it is not. They kept the excised heart of a frog beating normally for thirty-three days, giving about 360,000 beats, in Ringer's solution containing glucose and oxygen only, in addition to the salts. Any store of protein, if used for energy purposes, must have been exhausted early in the experiment. If any was used, it could only have been the minimal amount required for repair of the machine. See also the work of Evans and Matsuoka (1915) as regards the relation of oxygen consumption to the production of energy.

Rohde found that the effect of adding atropine, or adrenaline, or retarding oxidation by potassium cyanide, is to increase the consumption of glucose and

(probably as a secondary effect) to diminish the relative amount of reserve material consumed. The sugar does not appear to be completely oxidised, since, especially under the action of cyanide, organic acids and aldehydes are formed. The effect of increase of carbon dioxide is interesting. Here there is no evidence of abnormal forms of oxidation of glucose, and yet the amount of pressure produced per unit of oxygen consumed is enormously depressed. The pressure produced, in fact, falls almost to zero, yet the oxygen consumed continues to be more than half that of the normal period. It seems that the chemical processes go on normally, but the change of chemical to mechanical energy is prevented by carbon dioxide.

The experiments of Mines (1913, 3) have shown the importance of $H^{\cdot}$ ion concentration as regards the spontaneous beat of the heart. He finds that there is an optimal concentration. Excess may abolish the mechanical change while the electrical change may remain intact. The absence of $Ca^{\cdot\cdot}$ ions has the same effect, as shown in Fig. 172 (page 539).

Yrs sincerely
W. H. Gaskell

FIG. 229. PORTRAIT OF GASKELL.

(From a negative by V. H. Mottram.)

THE ORIGIN OF THE HEART BEAT

Whatever may be the impulses that cause the heart to beat rhythmically in certain invertebrates, as in Limulus, where Carlson (1904) showed that they are given off by ganglion cells, there is no doubt that the heart muscle of the vertebrate is capable of rhythmic beats in the absence of nerves. This fact is shown most conclusively, perhaps, by the experiments of Burrows (1912), who showed that bits of heart muscle from embryo chicks continued to beat in blood plasma as long as thirty days, and that cells wandered off from the mass and continued to multiply. The newly formed cells then commenced to beat rhythmically. Similar facts are described by Margaret R. Lewis (1915) for skeletal muscle.

It was first definitely shown by Gaskell (1882) that all the phenomena observed in the hearts of the frog and tortoise are to be explained satisfactorily on the basis of a "myogenic" origin, that is, not only does the beat arise spontaneously in muscular cells, but also the conduction of the excitation from one part of the same heart cavity to another part of that cavity, and from one cavity to another, takes place by muscular tissues. A portrait of Gaskell will be found in Fig. 229. This view was taken up by Engelmann on the Continent, and powerfully supported by his work. Now, while there is no difficulty, as far as muscular continuity is concerned, in the case of the lower vertebrates, it was believed, until the work of Stanley Kent (1893) and of His (1893), that there is no muscular continuity between the auricles and ventricles of the mammal. These observers showed that there is a bundle of a special kind of muscle fibres at a particular place, putting the muscular structures of auricle and ventricle into direct connection. The anatomy of the "bundle of

His," as it is called, was further worked out by Tawara (1906), and it is almost universally accepted now that the transmission of excitation in the mammalian heart takes place by means of this muscular bundle. Kent (1893 and 1914) describes another conducting path between the right auricle and the external wall of the right ventricle; but its importance is doubtful. Laurens (1916) finds indications of differentiation into special conducting paths in the turtle.

The existence of nerve fibres in the heart muscle is sufficiently accounted for by the vagus and sympathetic supply. Whether there is any kind of transmission by a nerve network seems very questionable; the heart beat is certainly not initiated by periodic discharges of ganglion cells, and if a nerve network plays any part in the transmission of excitation, it must be one of that kind whose existence has never been proved in the higher animals (see pages 472-473 above). It must be, in fact, able to conduct equally in all directions. Beats can be obtained by stimulation at any point of the heart muscle, and auricular beats can be brought about by backward transmission from the ventricle.

As was shown by Gaskell, the automatic rate of each chamber, when it is beating by itself, is slower than that of the chamber preceding it in the cardiac cycle. Thus, in the frog and tortoise, the rate of the sinus is the quickest, that of the bulbus slowest, the order being sinus, auricle, ventricle, bulbus. The sinus is therefore the "pace-maker." The work of Schlomowitz and Chase (1916) localises the actual spot in the right sinu-auricular junction. In the mammalian heart there is no separate sinus. Where is then the "pace-maker"? An excellent account of the history of the work on this question will be found in the paper by Thos. Lewis (1913, 3). The main facts only can be given here. Keith and Flack (1907) found traces of sinus tissue in certain parts of the auricle, especially at the junction of the superior vena cava with the right auricle. At this point there is a collection of peculiar muscular tissue in intimate relation with the nerves entering the heart. The clearest proof that the normal beat arises in this "Keith-Flack node" has been given by Thos. Lewis (1910). The principle of the method used is simple, depending merely on the fact that muscle in excitation is electrically negative to that at rest. By taking a series of photographs, with the string galvanometer, of the electrical effects from electrodes placed on various points of the auricle, it was shown that the normal beat actually commences in this sino-auricular node. This place was found to become electrically negative before any other place. From it the excitation spreads in all directions. Confirmatory evidence that the beat takes its origin here is afforded by the effect of warming and cooling the node, which is to affect greatly the rate of the whole heart, whereas similar results cannot be obtained from any other part. There is also other evidence, although less conclusive. The work of Sansum (1912), on the shortened compensatory pause after extra-systoles, shows that the sino-auricular or Keith-Flack node behaves like the sinus of the frog.

When the sino-auricular node is put out of action in any way, the beats are initiated by a point in the auriculo-ventricular bundle of His. They are transmitted from this point in both directions, so that simultaneous contraction of auricle and ventricle results. The rate of discharge of this node is, normally, slower than that of the Keith-Flack node, so that the latter sets the pace. Some observations by Cushny (1912) suggest that there is an additional cause for the subordination of the auriculo-ventricular node. If the bundle of His be cut across on the auricular side of its node, the ventricle is cut off from the impulses arriving from the auricle. But it does not at once develop its own rhythm, and Cushny brings evidence to show that the node is normally kept in a state of diminished excitability, owing to fatigue, by the impulses reaching it from the auricle. A similar state can, in fact, be induced by artificial stimulation of the auriculo-ventricular node, and is not due to inhibition.

We must suppose that these nodes discharge when they have stored up something to a sufficiently high degree, and that, after a discharge, they are incapable of further discharge until a fresh quantity has been formed. The experiments of Gaskell on the effects of clamping the auriculo-ventricular junction seem to show that the local effect of the clamp is to depress the rate at which the capacity of the tissue to contract is recovered. When the clamp is gradually closed, at

a certain stage it is found that the ventricle only responds to every second or third beat of the auricle. The cause must be at the part clamped, and due to the fact that a second impulse arrives before the tissue has recovered from the previous contraction. It is not easy to see how this could happen if the tissue were merely a conductor of excitation, since mere conduction would not leave the tissue in so pronounced a state of inexcitability.

Lewis (1915, 1) gives the following summary of the course of the excitation wave in the dog's heart. Starting from the sino-auricular node, it spreads in the auricle in all directions, finally arriving at the auriculo-ventricular node, where it is delayed. It then passes along the bundle of Purkinje tissue and is distributed to all parts of the ventricular muscle by the branches of this tissue, which conducts much more rapidly than the muscle itself. The advantage is a more simultaneous contraction of the whole of the ventricle. The actual conducting tissue consists of striated muscle, containing large amounts of glycogen. The following are the rates of conduction in the three different tissues concerned:—

Purkinje tissue - -	3,000 to 5,000 mm. per second.
Auricular muscle - -	1,000 mm. per second.
Ventricular muscle - -	300 to 500 mm. per second.

In the toad, Lewis (1915, 2) finds that the excitation spreads along the interior of the ventricle and passes out radially to the surface. The general direction of travel is thus from base to apex, but, at the surface, the extreme base is usually activated somewhat later than the apex.

Electro-cardiogram

The electrical change in the heart muscle has been discussed in the preceding chapter, and it is sufficient to refer here to the value of the electro-cardiogram as a method of investigation. We have seen above how it was used to determine the site of the pace-maker, and it is of equal value in detecting irregularities of transmission from auricle to ventricle, especially as they occur in disease.

The Coronary Circulation

The hearts of the higher vertebrates have, as would be expected, effective means of supply of oxygen by arteries which arise from the commencement of the aorta. The factors which influence the flow through this "coronary" circulation have been investigated by Markwalder and Starling (1913), using the method of Morawitz of introducing a canula into the coronary sinus. They found that the heart is insufficiently supplied with oxygen if the aortic pressure is lower than about 90 mm. of mercury, an important fact to be remembered in experimental work. The most potent agents in increasing the rate of flow are non-volatile metabolites produced by the heart muscle itself in its activity. These substances are present in considerable amount in asphyxia; in fact, the maximum circulation through the coronary vessels is just when the heart fails. Adrenaline causes dilation, probably by its action in increasing the rate and strength of the beats. The amount of blood flowing through the coronary vessels is very considerable, much more than had been supposed by previous workers. We shall have occasion to return to the question of the action of metabolites on blood vessels later. Since their concentration becomes so much greater in asphyxia, it seems that they are such products of activity as are normally removed in oxidation processes: lactic acid naturally occurs to the mind. The products of partial combustion of carbohydrates, as in Rohde's experiments (page 678 above), may also play a part. We have seen (page 611) that the anaerobic products of cell metabolism, however, are not necessarily the same as the intermediate products of normal oxidation.

Methods of Investigation

In recording the movements of the heart cavities, each for itself, the myocardiograph of Cushny (1910, 1) is very useful. For determining the intra-

ventricular or aortic pressure, Piper's modification of Frank's apparatus, already referred to, is the most accurate. The changes in volume of the heart are followed

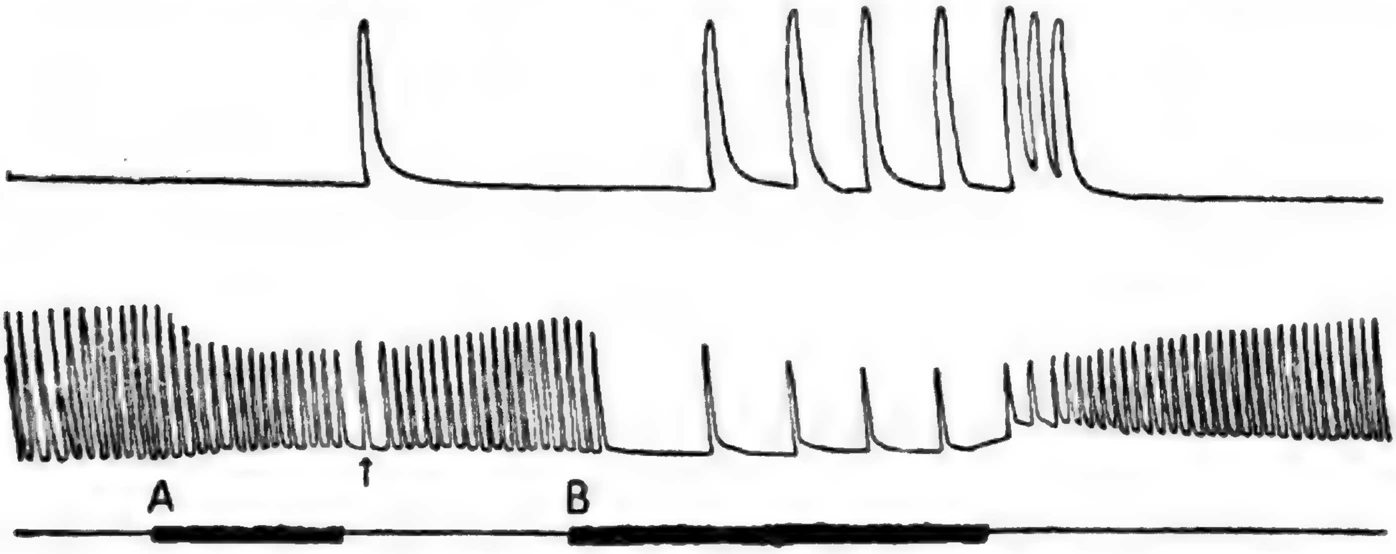


FIG. 230. EFFECT OF VAGUS STIMULATION UPON THE AURICULAR BEATS AND UPON HEART BLOCK.

Upper tracing, ventricle of turtle, at rest owing to presence of clamp on auriculo-ventricular groove.
Lower tracing, auricular beats.
The effect of vagus stimulation at *B* is to slow the auricular rhythm, and thus the conducting tissue is enabled to transmit the wave of excitation to the ventricle.
At *A*, with weaker stimulation, where the rate is unaffected, but the strength diminished, the ventricle remains at rest.

(Garrey, 1912, p. 456.)

by some form of plethysmograph, such as the glass cardiometer of Jerusalem and

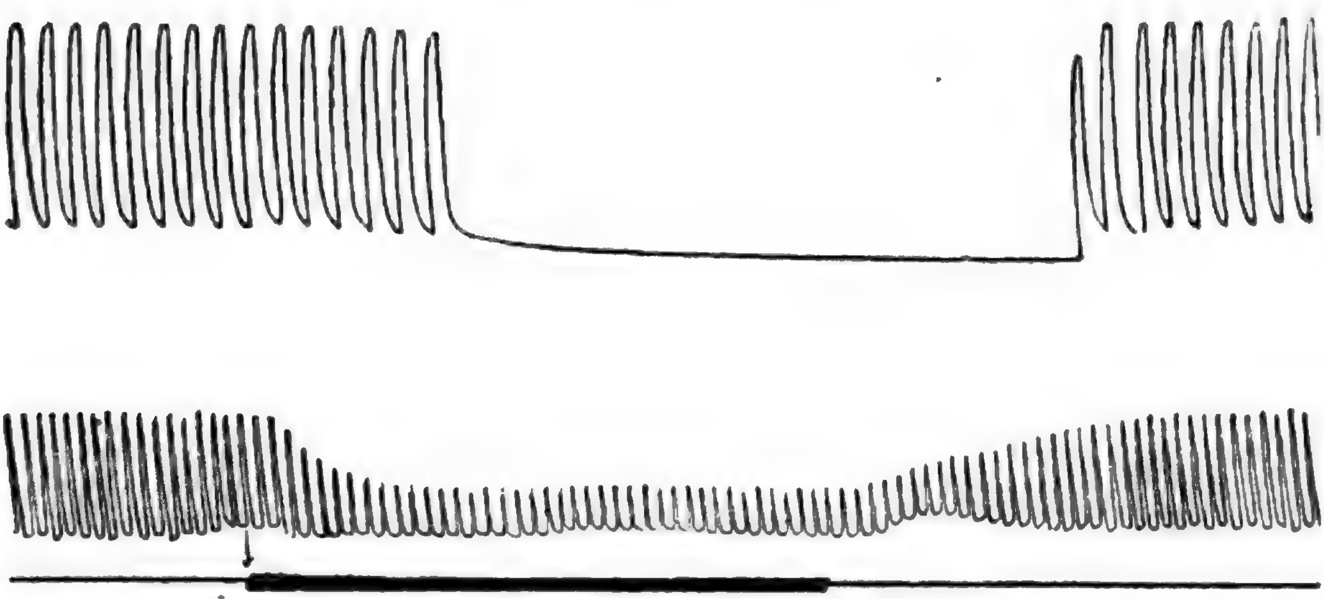


FIG. 231. EFFECT OF VAGUS ON CONDUCTION.—The ventricle, owing to auriculo-ventricular clamp, follows only each alternate beat of the auricle. Stimulation of the vagus causes complete block, together with diminution in size of auricular beats, without change of rate.

(Garrey, 1912, p. 454.)

Starling (1910). The output of blood may be recorded by the method described by Ishikawa and Starling (1912).

THE NERVOUS REGULATION OF THE HEART BEAT

Inhibition.—The discovery by the brothers Weber of the fact that the heart can be stopped by stimulation of the peripheral ends of the vagus nerves was of such fundamental importance that a few words as to its history are required. It was at the meeting of Italian Scientific Investigators at Naples in 1845

that Ernst Heinrich Weber made the announcement that he, with his brother Eduard, had found this to take place. As Tigerstedt (1893) remarks, a new kind of nervous action was brought to light, of which previously there had been scarcely any idea, an action which could never have been discovered by anatomical observations alone. A nerve, which supplied a muscle, was stimulated, and instead of strengthening or accelerating the movements of the muscle, they were slowed or stopped altogether. The original communication is, unfortunately, published in an Italian medical journal difficult of access (Omodei's *Annali di Medicina*). But in 1846 Eduard Weber wrote an article, "Muskelbewegung," for Wagner's "Handwörterbuch der Physiologie," which included an account of the work. It was found that stimulation of the vagus nerve inhibited the heart, not only in the frog, but also in fish, birds, the cat, dog, and rabbit. It is well to transcribe the words used: "Eine auf das Mannichfaltigste abgeänderte Reihe von Versuche, welche ich gemeinschaftlich mit meinem Bruder Ernst Heinrich aufgeführt habe, hat uns zu der Entdeckung geführt, dass durch Reizung der Nervi vagi oder der Hirntheile, von dem sie entspringen, das Tempo der rhythmischen Bewegungen des Herzens verlangsamt und sogar das Herz ganz zum Stillstand gebracht wird" (p. 42).

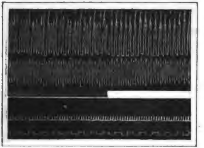


FIG. 232. ACTION OF VAGUS ON CONDUCTION FROM AURICLE TO VENTRICLE.—Dog's heart.

Upper curve, beats of ventricle.
Lower curve, beats of auricle.
Upper signal, stimulation of vagus.
Middle signal, artificial stimulation of auricle by single shocks, five per second.
Lower signal, time in seconds.
Before the stimulation of the vagus, the ventricle follows each auricular beat, with an occasional omission.
During stimulation of the vagus, it responds to each alternate beat only; showing that the conducting power of the tissue is impaired.

(Bayliss and Starling, 1892, 2.)

Since the work of the Webers, a large number of investigators have taken up the question of the action of the vagus on the heart, but we do not yet know what the nature of inhibition is. The problem has been discussed in some preceding pages of the present work (pages 418-427). I wish here to draw attention to some aspects of it as affecting the heart, referring the reader to the article by Gaskell (1900) for further details up to the date of the article.

As Gaskell pointed out (1882 and 1884), the action of the vagus is shown under four aspects, the names of which are due to Engelmann (1899):—

1. On the rate ("chronotropic"). This effect is shown in Figs. 113 (page 405) and 230.
2. On the strength of the beat ("inotropic"). Shown in the auricular tracing of Fig. 231.
3. On the capacity of the muscle for conducting excitation ("dromotropic"). Shown for the turtle in Fig. 231, and for the dog in Fig. 232 (see the descriptions of the figs.).
4. On the excitability to direct stimulation ("bathmotropic"). MacWilliam showed that the heart muscle, in some cases, is inexcitable under vagus stimulation (see page 407 above).

How far these different effects are aspects of the same fundamental change is not definitely known, but it seems probable.

In the mammal, Cohn and Lewis (1913) have shown that the left vagus has more effect on the junction between auricle and ventricle than the right one has. On the other hand, the right nerve affects production of impulses in the auricle more than the left one does. The facts are of interest in connection with what we have seen as to the facts of impulse formation in the sino-auricular

node, together with the conducting function of the tissues in which the auriculo-ventricular node is situated. The former is at the mouth of the superior vena cava, and the latter at the opening of the coronary sinus, which are the representatives, respectively, of the right and left ducts of Cuvier of the embryo. Thus, the right and left vagi act preferentially, each on that structure with which it would naturally be expected to be in morphological relationship.

Cohn and Lewis are of opinion that these results favour the view that the

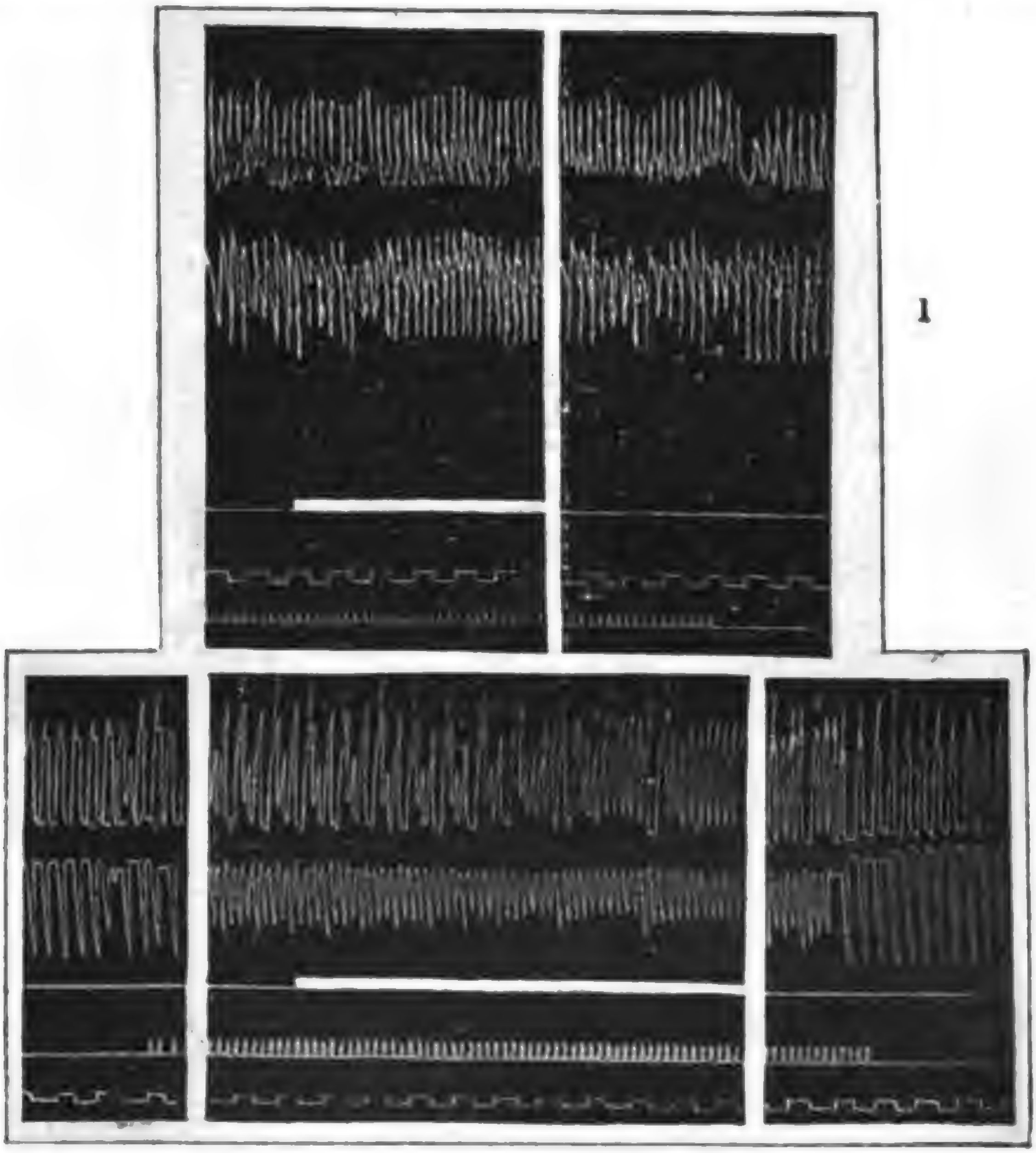


FIG. 233. ACTION OF ACCELERATOR NERVES IN IMPROVING CONDUCTION.

Upper curves, ventricle beats. Lower curves, auricle beats.

1, Ventricle stimulated at the rate marked by the lowest signal, namely, 22 shocks in five seconds. The auricle does not follow until the accelerators are stimulated as shown by the top signal. At the end of the tracing the artificial stimulation of the ventricle is stopped, and the normal rate of the beat is seen (17 in five seconds). Time in seconds given by the middle signal.

2, At the beginning the natural rate of the heart beat is seen (14 in five seconds). During the period shown by the middle signal, the auricle was stimulated at a rate of 25 in five seconds. The ventricle is unable to follow, until the accelerators are stimulated (top signal). Time in seconds by bottom signal.

(Bayliss and Starling, 1892, 2.)

particular aspects of vagus action, obtained reflexly by Engelmann, depend on the particular attributes of the tissue in which the fibres end, rather than on different functions of the same muscle.

Thos. Lewis (1914) further finds that the effect is more profound upon the auriculo-ventricular node than upon the sino-auricular node.

Some important conclusions as to the mode of action of the vagus on the ventricle are to be drawn from the work of Mines (1914) on the frog's heart. It is shown that atropine, applied to the sinus, eliminates the action of the vagus

on the rate of the beat, while the effect on the auriculo-ventricular junction and the ventricle remain. In these conditions the vagus decreases the rate of transmission from auricle to ventricle, and diminishes the duration of the state of excitation in the muscle fibres of the ventricle. This is associated with weakening of contraction in all parts of the ventricle, and not with failure of some of them to get excited at all. This diminution of duration of excited state by the vagus explains why it changes the sign of the final T-wave in the electro-cardiogram, if we admit that the negativity of the base indicated by this wave is due to the greater duration of the excitatory state at the base than at the apex. The action of the vagus being more powerful at the base than at the apex, it has the effect of reducing the duration of the excited state at the two to approximate equality, and thus tends to produce a simple diphasic effect. The application of atropine to different parts of the ventricle, by which the vagus endings are paralysed locally, confirms the conclusions arrived at.

Augmentor Nerves.—Like smooth muscle, the heart is supplied with two kinds of nerve fibres, inhibitory and excitatory. The latter were discovered by Von Bezold (1863), and their existence proved in a convincing manner by Von Bezold and Bever (1867). A tracing of their effect on the heart of the toad is given in Fig. 114 (page 406). In general, it may be said that the effect of the accelerator nerves is exercised on the rate and the strength of the beat, and on the conducting power of the muscle and, in all cases, in an opposite direction to that of the vagus. Fig. 233 shows the improvement of conduction. Increase of excitability has not, so far as I am aware, been demonstrated directly.

These nerves arise from the sympathetic system. Their antagonistic relation to the vagi has been discussed above (page 407).

Reflexes to Heart Nerves.—Both the vagus and accelerator nerves can be excited reflexly. It is not yet definitely known whether there is reciprocal innervation, such as that of the vasomotor reflexes, in the reflexes to the heart. Whether, for instance, when reflex slowing of the heart is produced, there is, along with excitation of the vagi, inhibition of tone of the accelerator centre. Bainbridge (1914 and 1915) finds that reflex acceleration of the heart is produced by inhibition of vagus tone, together with excitation of accelerators, but could find no evidence of inhibition of accelerator tone in reflex slowing. Possibly there was no tone in the accelerator centre, although the heart was apparently beating at a maximal rate. This may, however, have been from its own pace-maker, when relieved from vagus control.

The heart reflexes are closely interconnected with those to the blood vessels, and further facts with regard to them will be referred to along with the latter.

THE BLOOD VESSELS

Exact investigation of the phenomena of the circulation was impossible until Ludwig (1847) invented the graphic method of recording blood pressure. His portrait will be found in Fig. 234.

The Pulse Wave

Owing to the necessity of the pump being rhythmically active, the fact that the blood vessels possess elastic walls has considerable importance. Since the blood cannot pass through the arterioles as rapidly as it is driven into the aorta, if the arteries were rigid and unable to accommodate, temporarily, the excess of blood driven in by the contraction of the heart, it is plain that the efficient action of the heart would be put to great strain, owing to the incompressibility of the blood. Moreover, much less blood could be expelled at each beat. The result of the stretching of the arterial wall by the beat of the heart, owing to the elastic reaction when the aortic valves are closed, is to convert the rhythmic flow from the heart into a continuous one through the capillaries.

A further necessary incidental result of the elasticity of the arterial wall is the *pulse wave*. As the first portion of the system is distended by the blood, it

tends to close again, having, in fact, a certain period of vibration. This distension, therefore, disappears at one point and passes on to the next at a rate depending on this period of vibration. Details of the form of the pulse wave have not sufficient general interest to warrant description here, although they have considerable practical importance, as indicating states of the heart and blood vessels. It must be clearly understood that the wave of distension travels along the arteries at a much faster rate than the blood current itself. That the progression of a wave is independent of the current of the fluid itself is well

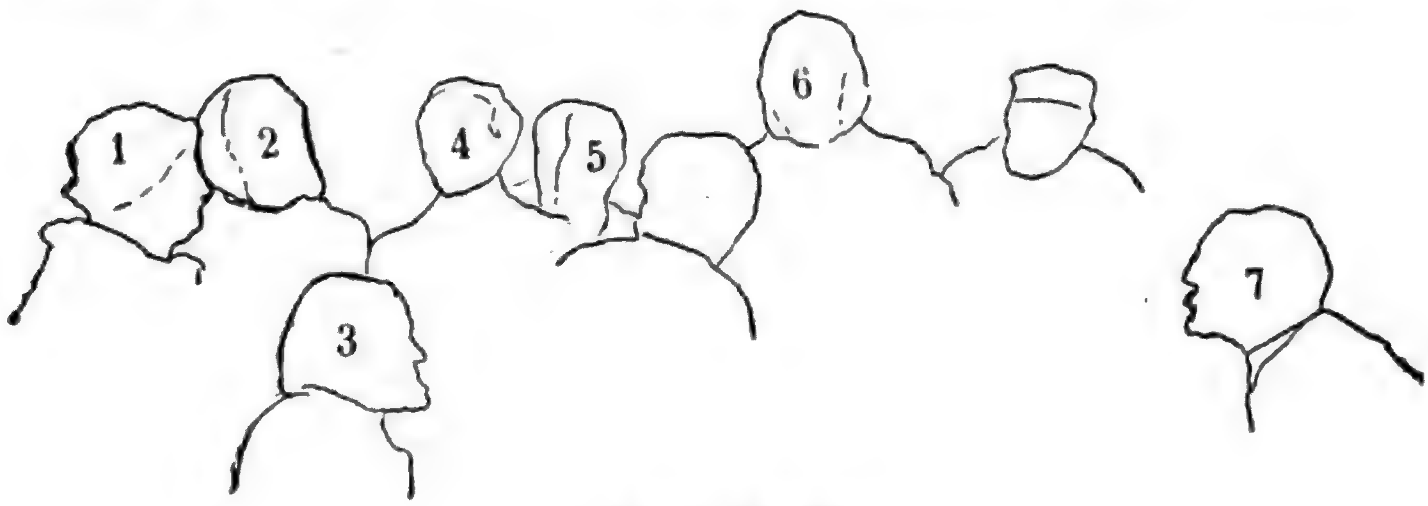
FIG. 234. PORTRAIT OF CARL LUDWIG.

shown by observing a sea-gull floating on a fairly calm sea. It will be seen that it merely rises and falls as the waves pass under it, without any change in its position otherwise.

FIG. 235. CLAUDE BERNARD DEMONSTRATING AN EXPERIMENT TO HIS FRIENDS.—The experiment seems to be the effect of the sympathetic on the rabbit's ear. Print copied from the picture by Lhermitte in the Luxembourg Gallery at Paris. The figures are

Hürthle (1912 and 1913) believes that he has found evidence that there is an active

contraction of the arteries at the latter part of each systolic wave, so that the blood is forced onwards by it. On the assumption that the rate of the current of blood through a certain length of artery is proportional to the difference of pressure between the two ends, it was found, under certain conditions, that the rate of the current was *greater* towards the end of the systolic part of the pulse wave than corresponded to the difference of pressure at this time. It is also stated that the amplitude of the pulse wave, instead of decreasing towards the periphery, as it does in the dead animal, is increased in the living animal, especially when the blood vessels are constricted. The interpretation of the facts is difficult, and further investigation is required. There is, of course, the possibility that the muscle of the arterial wall may respond to distension by a contraction, as that of the earthworm and the frog's stomach does (page 436). This idea is supported by some observations by Carl Tigerstedt (1913), who found an electrical change in the carotid artery with each heart beat. The direction of the deflection was such as to imply that the electrode nearest the heart became negative before the more distant one (see below, "Reactions to Changes of Pressure").



KEY TO FIG. 235.

1. M. Gréhant. 2, M. Dumontpallier. 3, M. Malassez. 4, M. Paul Bert. 5, M. D'Arsonval 6, M. Claude Bernard. 7, M. Dastre.

THE PERIPHERAL RESISTANCE

The nature of this, as conditioned by the internal friction of the blood, has been explained above (pages 241-242). The blood of the dog has a viscosity about five times that of water. How far changes in this property occur in physiological conditions and their effect on the blood pressure have not received much attention. According to Burton-Opitz (1911), the viscosity of normal blood is much higher than that of defibrinated blood. Deep narcosis with ether increases the viscosity, which falls again as the narcosis is diminished. Carbon dioxide also increases the viscosity; hence venous blood, in addition to the effect of loss of water, has a slightly higher viscosity than arterial blood. The viscosity, also, as would be expected, increases with the number of corpuscles per unit volume; and that of laked blood is less than that of the same blood before laking. Of course, dilution of the blood, as happens after loss of blood, has a considerable effect in reducing the internal friction (see also Denning and Watson, 1906).

The general effect of the existence of the peripheral resistance is to enable the heart to produce a high arterial pressure, with the advantages as regards regulation of blood supply to organs following therefrom, as already pointed out. It is scarcely necessary to say that the peripheral resistance must not be stated to be the cause of the blood pressure, which is due to the energy produced by the muscular contractions of the heart.

THE VOLUME OF THE BLOOD.

Dreyer and Ray (1910, 1911) have shown that the volume of the blood in mammals is proportional to their surface, that is to the $\frac{2}{3}$ power of the weight, multiplied by a constant for each species of animal. D. T. Harris (1920) has investigated the methods used for estimating this volume, especially that of the dilution of a known volume of a non-diffusible dye injected into a vein.

Since the vascular system is a closed one, it is clear that the more blood there is in any one part, the less there must be in the remaining parts. The amount of blood driven out by the heart in each beat, that is, the amount sent through the organs to supply them with oxygen, etc., depends on the amount present in its cavities in diastole, so that if there is any accumulation anywhere in the periphery, the circulating blood is decreased and the blood pressure falls. This would be very marked if the enormous capacity of the capillaries were increased by some means, although the fact also plays a part in ordinary vasomotor effects, as a so-called "capacity" effect. Consideration of the capacity factor must not be overlooked in regard to its effect on blood pressure whenever vascular constriction or dilatation occurs. The importance of this factor forced itself into attention in the "wound-shock" of the late war, and led to the use of transfusion of blood or gum-saline in order to compensate for the blood held up in the capillary region, dilated by toxic products from injured tissues (see Bayliss, 1918, and page 706 below).

The Regulation of Blood Supply

If the arterioles of an organ are caused to dilate, the volume of the blood flowing through the organ is increased, and the pressure in the capillaries raised. Coincidently, the peripheral resistance is decreased, so that, if the region in which the dilatation occurs is a considerable fraction of the whole circulation, the aortic pressure falls, unless the heart beat is increased to compensate for it.

Methods of Investigation.—In order to measure the state of the circulation in an organ, we may take tracings of the changes in its volume, due to greater or less distension of its blood vessels, by some plethysmographic method.

In this method the organ is enclosed in an air-tight box, provision being made that the nerves and blood vessels are not compressed, and the interior of the box is connected to some instrument which records by its movement the amount of air sent into or removed from the recorder as the organ alters in volume. It has been suggested that changes in general venous pressure would interfere with the correct interpretation of the results. In actual fact, it has been found that experiments by the plethysmographic method and by determination of the actual rate of flow of blood give the same results. Of course, due account must be taken of changes in the general arterial pressure, which alters the rate of flow apart from local changes. If, for example, along with fall in arterial pressure, the organ decreases in volume, no information is obtained as to any active changes in the blood vessels of the organ itself. But, if the organ expands with a fall of arterial pressure, no other interpretation is possible than that its blood vessels have actively dilated. If the organ contracts, with a fall of arterial pressure, we cannot draw the conclusion that local vaso-dilatation is absent, because it may be too small to counteract the effect of the general fall of pressure in draining away blood. When a secretory gland or the kidney is investigated, due regard must be taken to the increase of volume produced by the fluid turned into the ducts. But this method cannot distinguish between arterial and capillary dilatation (Dale and Richards, 1918).

The second method is one in which the blood flow from the vein of an organ is estimated. If it comes in drops, they are allowed to fall on to a lever which actuates a signal, electric or pneumatic. If more copious, it may be measured in a "tipper," such as that described by Condon (1913), or in a siphon outflow recorder, such as that of Ishikawa and Starling (1912) or of Gunn (1913).

Details of various methods will be found in Frank's article (1913).

Vasomotor Nerves.—The supply of the smooth muscle of the arterioles by both excitatory and inhibitory nerves has been referred to above (page 403) in the general discussion of excitation and inhibition.

The name "vasomotor" should be applied to both classes, since both bring about movement of the vessel wall. Confusion is sometimes caused by using "vasomotor" as equivalent to "vaso-constrictor."

The first clear proof of the existence of vaso-constrictor nerves was afforded, independently, by Brown-Séquard (1852) and by Claude Bernard (1852). They found that the blood vessels in the region supplied by the cervical sympathetic nerve were constricted when the peripheral end of the nerve was excited.

Fig. 235 is a copy of a picture by Lhermitte in the Luxembourg Gallery in Paris. Claude Bernard is shown demonstrating to friends and pupils this effect on the ear of the rabbit; at least, this appears to be the nature of the experiment. At a later date (1858) Bernard proved the existence of vaso-dilator nerves by stimulating the chorda tympani nerve, and observing the greatly increased outflow from the vein of the submaxillary gland. The reader may, perhaps, find Fig. 236 of interest in connection with the great physiologist.

Most of the organs of the body have been shown to have a supply of both vaso-constrictor and vaso-dilator nerves. Fig. 110 (page 403) shows the dilatation produced in the tongue of the dog by the lingual nerve and the constriction produced by the cervical sympathetic nerve. Fig. 109 (page 402) is a copy of the figure by Retzius of the nerve plexus around arterioles.

à Monsieur le professeur Ludwig
hommage de l'auteur
Cl. Bernard.

SIGNATURE OF CLAUDE BERNARD.

(From a reprint sent to Carl Ludwig.)

FIG. 236. STATUE OF CLAUDE BERNARD IN PARIS.

Although nearly all the organs of the body are thus supplied with vasomotor nerves, it is remarkable that no experimental evidence of a satisfactory nature has been brought to show their presence in the *brain* (see Bayliss and Hill, 1895). It appears that the central nervous system is of such importance that, when more blood is required by it, this blood has to be provided by rise of arterial pressure, produced by vaso-constriction in the rest of the organism.

The *coronary arteries* of the heart are probably in a similar position. But here there appears to be no need for constrictors, since the heart is always at work, and increased supply is automatically provided by rise of aortic pressure and by the great sensibility of the coronary vessels to the metabolites produced by the heart in contraction.

For some time the supply of vaso-constrictors to the *lungs* was in doubt. Bradford and Dean described their presence, Brodie and Dixon denied it. Fuhner and Starling (1913) and Macht (1914) finally proved it by the action of adrenaline.

Space does not permit of details of the anatomical origin of the various supplies to individual organs. These will be found in my article (1906, 3), and in the papers quoted there. The vaso-constrictors are all contained in the sympathetic outflow, as described by Gaskell. The vaso-dilators have a more varied origin, especially from cranial and sacral visceral systems. The question of the existence of vaso-dilators in the sympathetic has been discussed above (page 428). Further discussion will be found in the paper by Hartman (1915). The fall of blood pressure produced under some conditions by small doses of adrenaline is perhaps the strongest evidence; but there is something peculiar about this fact, because, according to Hartman's experiments, the dilator effect is confined to peripheral vessels, absent from the splanchnic area.

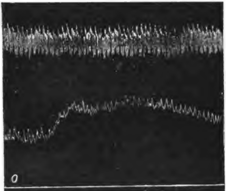


FIG. 237. VASO-DILATATION IN THE LEG ON STIMULATION OF THE FIRST SACRAL DORSAL ROOT.—Nine days after removal of the spinal cord from the second lumbar segment downwards, leaving intact the dorsal root ganglia. The ventral roots were completely degenerated and inexcitable.

Upper tracing, blood pressure.
Lower tracing, volume of the hind leg.
The upper, straight line is the zero of the blood pressure.
The lower, signal line marks the duration of stimulation.

(Bayliss, 1901, 2, p. 191.)

The remarkable nature of the vaso-dilator supply to the limbs, skin of the trunk, and, probably, of the ears and face, and of the intestine requires mention. Stricker had described vascular dilatation in the foot of a dog when he stimulated the peripheral ends of dorsal roots in the sacral region, but the statement was not generally accepted until my work (1901, 2), on account of the apparent contradiction of the law of Bell and Magendie (Fig. 237). I showed that the fibres concerned have anatomical relationships similar to those of the ordinary sensory fibres, and appear to be identical with them. I suggested that there might be a peripheral nerve network around the arterioles, common to both sensory fibres and vaso-dilator nerves. This view was confirmed by the work of Ninian Bruce (1910). Starting from the fact that paralysis of sensory nerves by cocaine prevents the application of oil of mustard from causing inflammation, he showed that the inflammation produced by this irritant is due to an axon reflex to the arterioles. It is unaffected by mere section of the nerve trunk, but disappears if the nerve fibres are allowed to degenerate. Thus a sensory fibre has a vaso-dilator branch to an arteriole, and, when the receptor organ is stimulated, the nerve impulse, as it reaches the branch, passes along it and causes dilatation of the arteriole (see the diagram of Fig. 145, page 474). It will be noted how the four classical characteristics of inflammation—heat, pain, redness, and swelling—fit in with this view: pain from the sensory component, heat, redness, and swelling from the

vascular dilatation. There are also vaso-dilators to the intestine in the dorsal roots (Bayliss, 1902, 3).

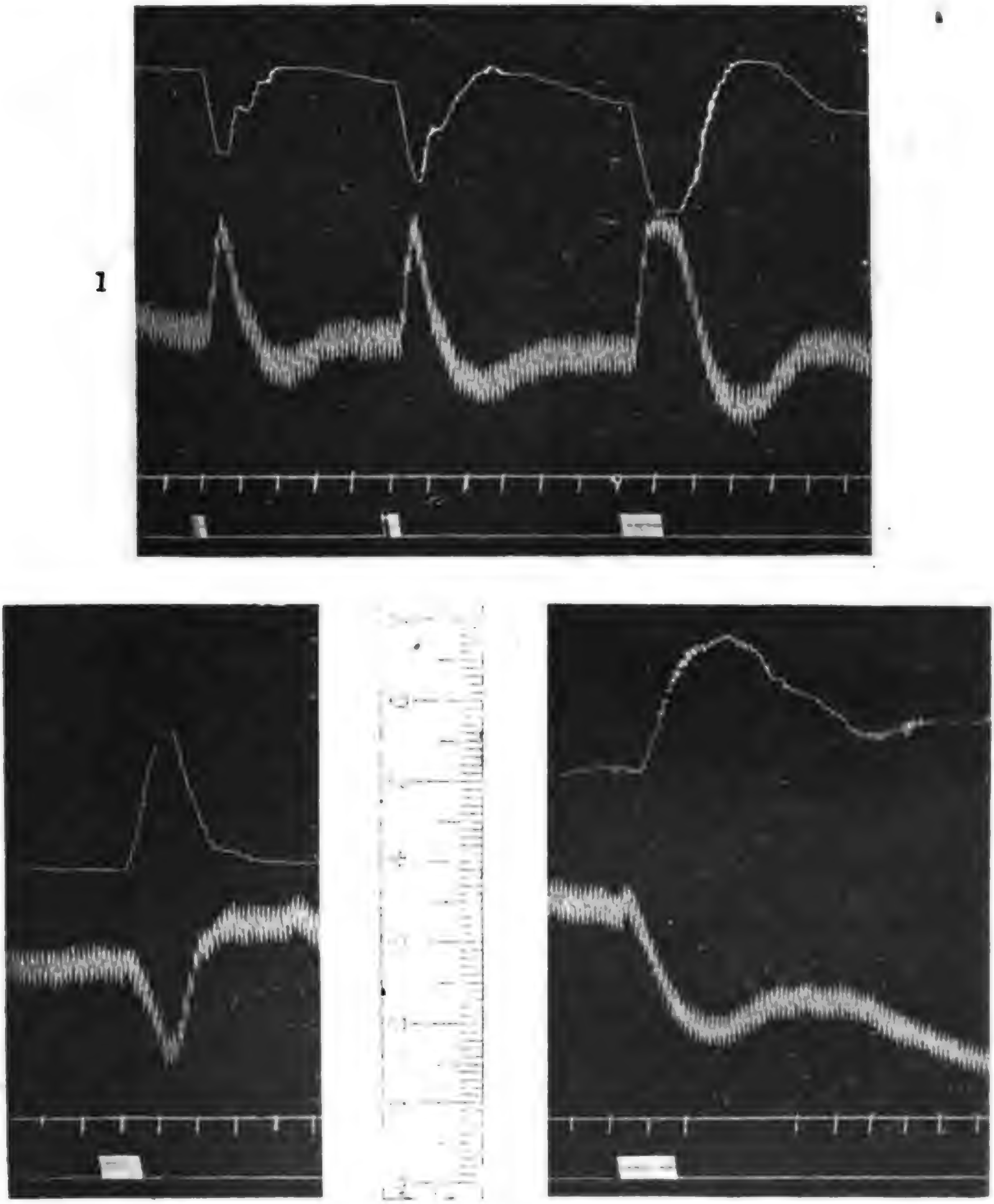


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FIG. 238. VASOMOTOR REFLEXES.

Upper curves in each fig., volume of intestine of cat, recorded by plethysmograph. Fall indicates arterial constriction.

Lower curves, arterial pressure in aorta. Mercury manometer. The initial normal height was 90 mm. mercury. Each division of the scale represents 2 mm. of mercury change of pressure.

Upper signal, time in ten-second intervals.

Lower signal, stimulation.

1, Stimulations, three in number, of the central end of the median nerve. Rise of blood pressure, caused by peripheral constriction, as shown by the volume of the intestine.

2, Stimulation of the central end of the vagus (depressor). Fall of blood pressure, caused by peripheral dilatation (increase of volume of the intestine).

3, Similar to previous curve. Showing effect of more prolonged stimulation in producing permanent fall of blood pressure, probably owing to secondary effect of anæmia on the heart.

The relation of these *antidromic* impulses, as I called them on Langley's suggestion, to herpes has been indicated above (page 290). Affections of the Gasserian ganglion may also be mentioned.

It is found in some cases that a nerve, which ordinarily causes vaso-constriction, may, after certain drugs such as ergotoxine (see the paper by Dale, 1906), produce dilatation in the same region. The obvious explanation is that usually given, namely, that the nerve contains both kinds of fibres, and that the constrictors are paralysed by the drug. Some effects of this kind have been discussed above, and it was pointed out that the possibility of the effect of the constrictor fibres being reversed by the drug has not yet been definitely excluded.

Vasomotor Reflexes.—General peripheral constriction or dilatation can be produced reflexly, with rise or fall of arterial pressure. There is one nerve, called the depressor, which always causes a fall and, under ordinary conditions, all other sensory nerves cause a rise. But, in certain states and modes of stimulation, reflex fall of blood pressure can be obtained from the latter. This fall appears to be due to a genuine vaso-dilator reflex (Ranson and Billingsley, 1916, 3 and 4), the afferent impulses travelling by a special path (see below).

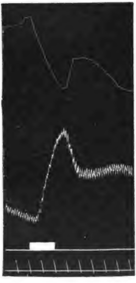

FIG. 239. EXCITATION OF VASO-CONSTRICTORS IN PRESSOR REFLEX.

Upper curve, volume of hind leg of cat.
Lower curve, arterial pressure. Zero, 20 mm. below time signal.
The vaso-dilator supply was cut off by previous section of the spinal cord in the middle of the lumbar region; that is, on the cranial side of the outflow of these nerves.
The central end of the median nerve (pressor) was stimulated at the time indicated by the upper signal.

(Bayliss, 1908, 2, Fig. 5.)

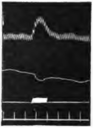

FIG. 240. INHIBITION OF VASO-DILATOR TONE IN PRESSOR REFLEX.

Upper curve, arterial pressure. Zero, 30 mm. below time signal.
Lower curve, volume of external ear of rabbit.
Vaso-constrictor supply cut off by section of the sympathetic nerve in the neck.
A rise of blood pressure occurs when the central end of the median nerve is stimulated. This is accompanied by diminution in volume of the ear, although the only vasomotor nerves left were dilators.
Further details will be found in the paper given below.

(Bayliss, 1908, 2, Fig. 6.)

The *depressor nerves* were discovered by Cyon and Ludwig (1866), who found that, in the rabbit, there is a branch of the vagus which proceeds to the heart and consists of afferent fibres from this organ. When the central end of this nerve is stimulated, with the main trunk of the vagus intact, a slowing of the heart beat is caused, accompanied by a fall of blood pressure. That the fall of blood

pressure is not entirely due to the cardiac inhibition, was shown by the fact that it was still present when the vagus nerves were cut, and no change in the heart beat occurred. Fig. 127 (page 423) is a typical form of depressor curve in the rabbit with vagi cut. The fall of blood pressure is, in fact, produced by general vaso-dilatation in all organs of the body which are supplied by vasomotor nerves. Fig. 238 shows that it occurs in the intestine, and Fig. 120 (page 412) that it occurs in the leg.

The peripheral receptor ends of the depressor nerve are situated not only in the heart but in the arch of the aorta, and its function appears to be to protect the heart from too great a rise of blood pressure. We see in Fig. 106 (page 386) how it is excited at each heart beat, but it has been found difficult to show experimentally that a rise of aortic pressure, produced otherwise than by each heart beat, stimulates it. In most mammals the depressor fibres are contained in the trunk of the vagus, and it is not always possible to obtain their effect apart from the opposite pressor effect of the ordinary sensory fibres in the vagus trunk. In the cat the left vagus usually acts as a depressor nerve only.

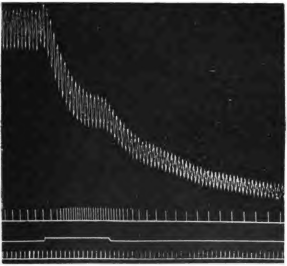

FIG. 241. EXCITATION OF VASO-DILATORS IN DEPRESSOR REFLEX.

Arterial pressure in cat, upper curve. Zero, 33 mm. below time signal.
Upper signal line, drops of blood from vein of submaxillary gland.
Middle signal, stimulation of central end of vagus.
Lower signal, time in two-second intervals.
Cervical sympathetic cut, so that the gland was supplied with vaso-dilator fibres in the chorda tympani nerve, and these were the only vasomotor nerves present.
The first part of the fall of pressure is accompanied by vascular dilatation in the gland, shown by the more rapid succession of drops. The later continued fall, after ceasing the stimulation, is, no doubt, due to failure of the heart from insufficient blood supply, and not to peripheral dilatation.

(Bayliss, 1908, 3, Fig. 1.)

Fig. 238 gives instances of a reflex rise and a reflex fall of arterial pressure, and the plethysmograph curve of the intestine shows that the rise is produced by a peripheral vaso-constriction and the fall by dilatation.

Now we have seen that there are both constrictor and dilator fibres leaving the central nervous system, so that there must be centres from which they arise.

It has long been known that there is a particular part of the bulb which acts as centre for the former, although it is probable that what is called the "vasomotor centre" is rather a nerve tract containing fibres from scattered centres.

Ranson and Billingsley (1916, 2) find that localised stimulation of a region of the fovea inferior in the floor of the fourth ventricle causes vaso-constriction, while that of a region of the area postrema, lateral to the obex, causes vaso-dilatation.

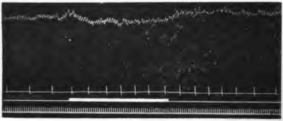

FIG. 242. Similar experiment to that of Fig. 241, but on the rabbit, the central end of the depressor nerve itself being stimulated.
There is very little fall of blood pressure, owing to the use of a mercury compensating arrangement.

(Bayliss, 1908, 3, Fig. 7.)

Porter and Turner (1915) hold that there are distinct centres for vascular reflexes, and for maintenance of vascular "tone."

The **afferent** spinal path for depressor reflexes lies in the lateral column in the same position as the spino-thalamic tract, but its fibres do not ascend beyond the bulb (Ranson and Billingsley, 1916, 3). The afferent path for pressor reflexes is in the tract of Lissauer. The fibres follow the same course as the protopathic pain fibres of Head (Ranson and Billingsley, 1916, 1).

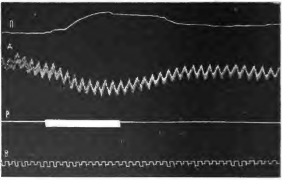

FIG. 243. VASCULAR DILATATION IN THE HIND LIMB BY EXCITATION OF DILATORS.—Abdominal sympathetic cut.

n, Volume of hind leg of dog.
A, Arterial pressure.
p, Signal marking stimulation of central end vagus.
B, Time in seconds.

Coincident with the fall in arterial pressure, due to the depressor fibres in the vagus, there is dilatation of the limb, although there were no vaso-constrictors whose tone could be inhibited.

(Fofanov und Chalussov.)

It is evident, then, that reflex vaso-constriction or dilatation might each be produced in two ways; the former, either by excitation of the constrictor centre or by inhibition of tone in the dilator centre, supposing such tone to be present; the latter, by excitation of the dilator centre, or by inhibition of tone of the constrictor centre, supposing it to be in a state of tonic excitation.

In early work on vascular reflexes, the existence of vaso-dilator nerves was not taken account of, so that the peripheral dilatation produced from the depressor nerve was ascribed by Cyon and Ludwig to inhibition of the constrictor centre. In 1893, in the course of investigations with this nerve,

I found that the phenomena were not to be satisfactorily explained on this view alone, and suggested (page 317) that, both in pressor and in depressor reflexes, inhibition of the one centre is associated with excitation of the opposite one.

Ostroümov, working with Heidenhain (1876), had already expressed the view

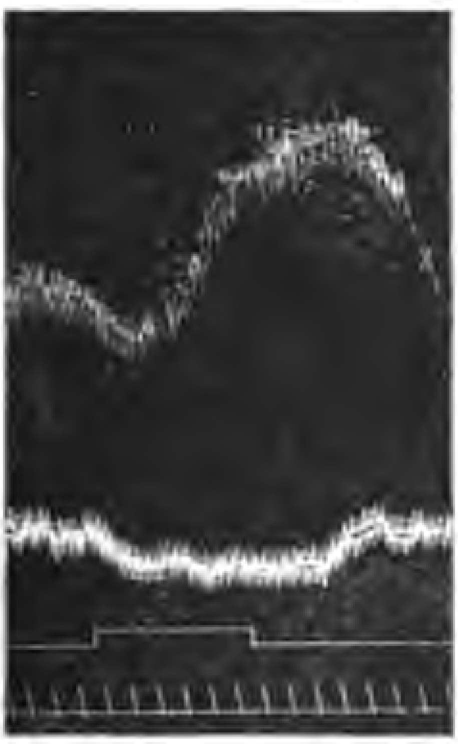

FIG. 244. Experiment similar to that of the preceding figure.

Upper curve, volume of hind leg of dog.
Lower curve arterial pressure. Zero, 23 mm. below signal.
Signal marks stimulation of central end of vagus.
Time in ten-second intervals.
The reflex dilatation obtained after section of the vaso-constrictors in the abdominal sympathetic can be abolished by subsequent section of the spinal cord at the second lumbar nerve, by which operation the connection of the vaso-dilators with the centre is severed.

(Bayliss, 1902, 3, Fig. 7.)

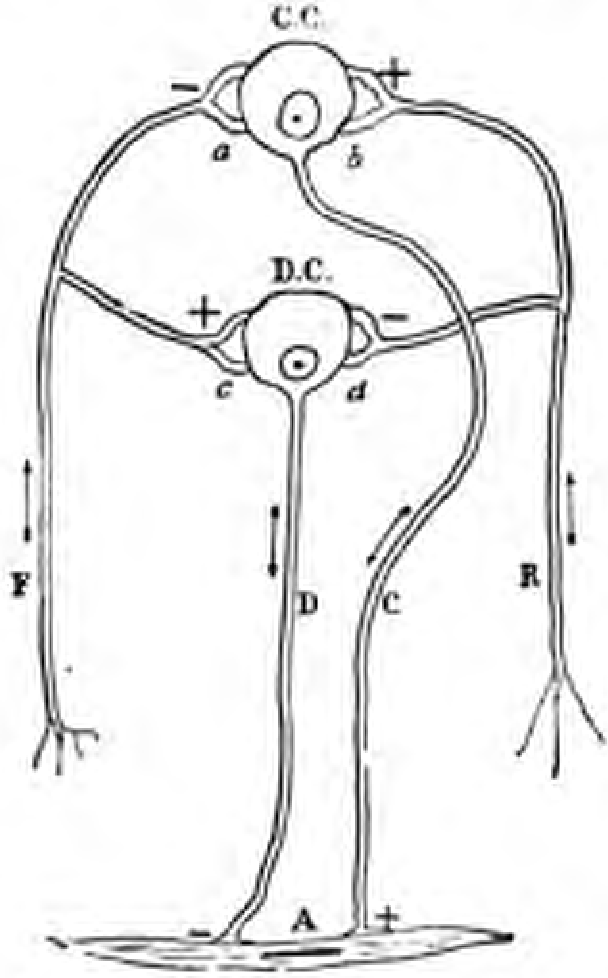

FIG. 245. DIAGRAM OF THE ARRANGEMENTS OF VASOMOTOR REFLEXES.

A, Muscle cell of arteriole.
D, Vaso-dilator nerve fibre terminating on *A*, and inhibiting its natural tonus.
C, Vaso-constrictor fibre also ending in *A*, but exciting it.
These two kinds of fibres arise from the dilator centre (*D.C.*) and the constrictor centre (*C.C.*) respectively.
F, Afferent depressor fibre, dividing into two branches (or collaterals), one of which (−) inhibits the constrictor centre, while the other (+) excites the dilator centre.
R, Pressor fibre of ordinary sensory nerve, causing rise of arterial pressure by exciting *C.C.* and inhibiting *D.C.*
a, *b*, *c*, *d*, The respective synapses of these branches with the efferent neurones.
The probable intermediate neurones are, for the sake of simplicity, omitted.

(Bayliss, 1908, 2, Fig. 27.)

that the reflex dilatation in the skin vessels produced by the depressor nerve is due to excitation of vaso-dilator fibres, but it does not seem to have occurred to these and subsequent investigators that *both* excitatory and inhibitory actions are concerned. After Sherrington's work on the reciprocal innervation of skeletal muscle, I took up the question again (1908, 2), and showed that this mode of innervation applies also to vasomotor reflexes, although, of course, the phenomena are complicated by the fact that both the centres and the effectors can be excited or inhibited. There are thus four cases to be considered, which must be done

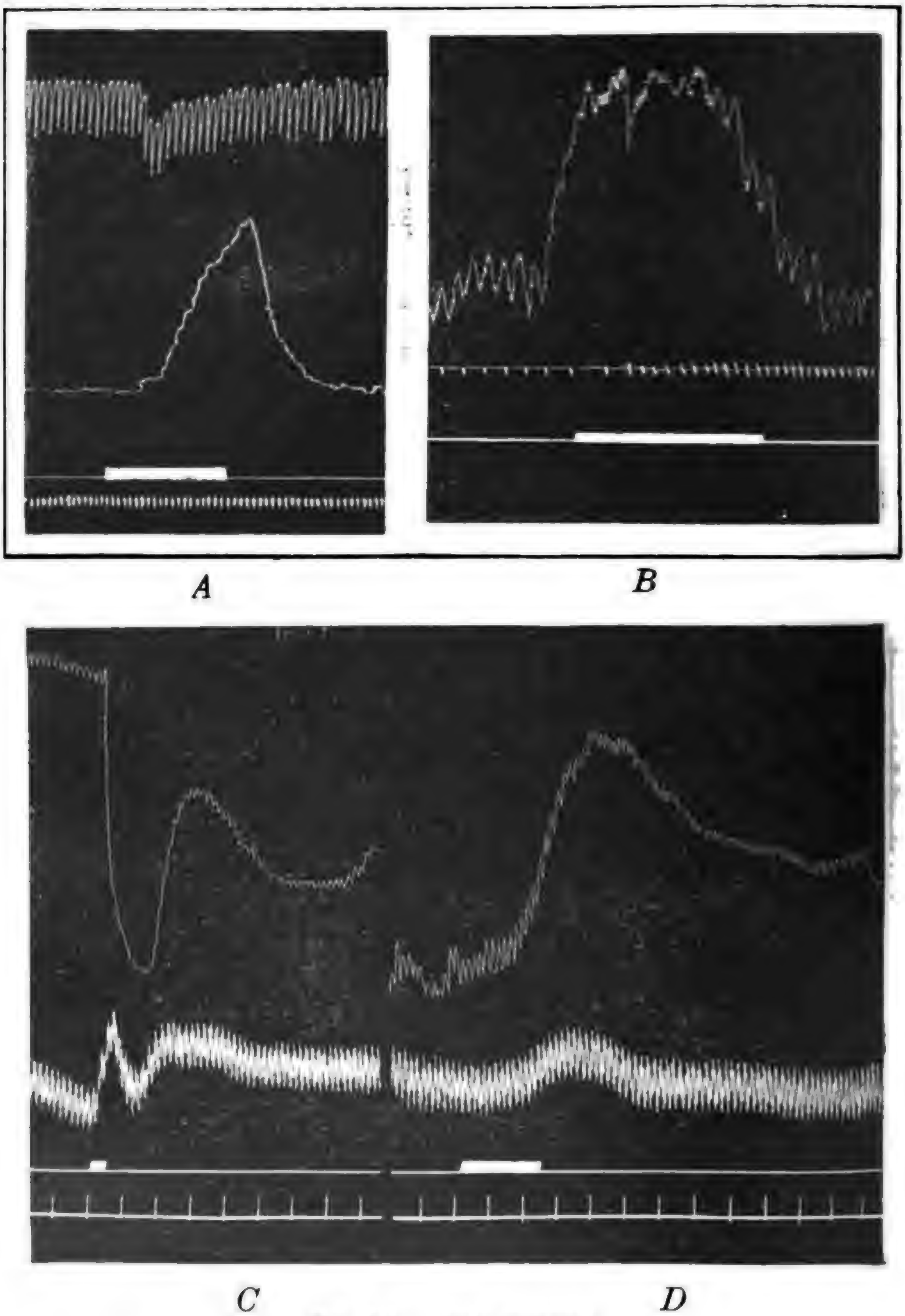

FIG. 246. LOVÉN REFLEXES.

A, Upper curve, blood pressure.
Lower curve, volume of upper part of hind leg of dog.
At the signal, the central end of the dorsalis pedis nerve of the same leg was stimulated, causing a slight fall of blood pressure, with marked dilatation of the leg.
Note that the usual effect of a sensory nerve is to cause reflex vaso-constriction in the body generally.
B, Upper curve, blood pressure.
Lower curve, drops of blood falling from cut femoral vein.
At the signal the central end of the anterior crural nerve of the same leg was stimulated. A rise of blood pressure is seen, accompanied by dilatation of the leg. Fig. 239 shows that stimulation of a sensory nerve from another region, namely, the median of the arm, causes vaso-constriction in the leg. This fact is also shown in
C, Upper curve, volume of hind limb of dog.
Lower curve, blood pressure.
Vaso-dilators cut off by section of the lumbar and sacral dorsal roots.
At the signal, the central end of the median nerve was stimulated.
The rise of pressure is accompanied by constriction in the leg.
D, Same experiment, but instead of the median nerve, the central end of a sensory root of the leg area, namely, the sixth lumbar dorsal root, was stimulated.
Again there is the usual rise of general blood pressure, but the vessels of the limb itself dilate considerably.
This experiment shows that in the Lovén reflex there is inhibition of constrictor tone, and the proof is given in Fig. 9 of my paper of 1902, 3, that the dilators are excited, so that reciprocal innervation holds in this case.

(*C* and *D* from Bayliss, 1908, 2, Fig. 8.)

briefly here; for further evidence the reader is referred to my two papers (1908, 2 and 3).

In *pressor reflexes* we have rise of arterial pressure produced by contraction of peripheral vessels. This is due, mainly, to excitation of the constrictor centre, which sends impulses causing the smooth muscle of the arterioles, already in a state of moderate tonus, to contract still more. Fig. 239 shows that this takes place, because the leg was supplied only by vaso-constrictors, the dilators having been cut. Figs. 240 and 124 (page 416), however, show that constriction can be detected in pressor reflexes when the organ under investigation is supplied only with dilator fibres. In such cases it must be due to the fact that the muscle of the arterioles was kept in a state of inhibition by impulses from the dilator centre, and that the afferent impulses which excite the constrictor centre also inhibit the dilator centre, thus removing the tonic inhibitory influence on the blood vessels, and allowing them to return to their normal state of tone. It is to be remembered that, as is pointed out by Sherrington, inhibition cannot be detected unless there is tonic excitation to be removed. It is, in fact, only in certain states favourable to tonic vascular dilatation, such as high temperature of surroundings and high blood pressure, that it is possible to detect inhibition of dilator tone, apparently on account of the absence of tone in ordinary conditions (see also Fig. 248 below).

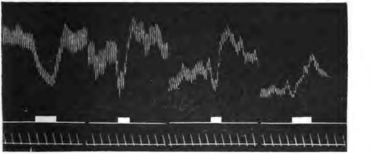

FIG. 247. EFFECT OF STRYCHNINE IN CONVERTING THE DEPRESSOR FALL IN THE RABBIT INTO A RISE OF PRESSURE.

The first stimulation is of the normal nerve.
A dose of strychnine was given between each stimulation.
The fall is gradually abolished and its place taken by a rise.

(Bayliss, 1908, 2, Fig. 15.)

When we turn to *depressor reflexes*, we find it quite easy to show that the constrictor centre is inhibited. Fig. 120 (page 412) shows this, since the dilators had been cut. Excitation of dilators is shown in the case of the chorda tympani nerve, which contains no constrictor fibres, in Fig. 241. Since, however, this nerve contains secretory fibres, which might be supposed to be excited from afferent fibres in the vagus nerve, although no secretion was observed, objection might be taken to this interpretation. Metabolites might be formed by the cells, and these, as we have seen, cause dilatation of the blood vessels. Fig. 242 is, therefore, more convincing, being the reflex from the depressor nerve of the rabbit, which cannot be held to cause reflex secretion.

Fofanov and Chalussov (1913) have confirmed these results, and show that vaso-dilatation in the tongue, the nasal mucous membrane, and the legs occurs when the central end of the depressor is stimulated, although the vaso-constrictor supply has been cut off. Fig. 243 is a copy of one of their curves. The case of the leg is interesting, as I had already pointed out (1902, 3, p. 292), on account

of the fact that no dilators, other than the dorsal root fibres, can be detected, so that these must be excited from the centre in an opposite direction to that which would be considered to be the normal one. Fig. 244 is from an experiment of my own, also showing this fact.

Martin and Mendenhall (1915) have shown that there is vaso-dilatation in the nasal mucous membrane when the depressor is stimulated, although the vaso-constrictors were cut.

In Fig. 245 a diagram of the connections of the two vasomotor centres is given; this may assist in following the somewhat complex state of affairs.

Sherrington (1913, 2, p. 93) interprets these phenomena as being allied to the postural tonus of skeletal muscle. The automatic tonus of the arterial wall is postural as regards the contained blood. As it is only under special conditions that flexor tonus can be obtained, so it appears that tonus of the vaso-dilator centre is not usually to be detected.

FIG. 248. VASO-CONSTRICTION BY INHIBITION OF DILATOR TONE. — Ear of rabbit. Sympathetic cut.

Upper curve, volume of ear.
Lower curve, blood pressure.
At the signal, the central end of the median nerve was stimulated. There was only a slight rise of blood pressure, because the animal had been eviscerated. Constriction of the ear is shown, preceded by slight dilatation.

(Bayliss, 1908, 2, Fig. 22.)

FIG. 249. INHIBITION OF DILATOR TONE CONVERTED BY STRYCHNINE INTO EXCITATION OF DILATORS.

Same experiment as in Fig. 248, but after the injection of strychnine.
Stimulation of the median nerve caused *dilatation* of the ear, accompanied by a slight fall of arterial pressure.

(Bayliss, 1908, 2, Fig. 23.)

Lovén Reflexes.—An interesting form of local vascular reflex was first described by Lovén (1866). When the central end of the great auricular nerve, the sensory nerve of the ear in the rabbit, was stimulated, it was noticed that, although vaso-constriction was produced in other organs, in the ear itself vaso-dilatation occurred. A similar effect was obtained in the leg. Fig. 246 gives two tracings of this latter effect. It appears, thus, that an active organ may bring about a better blood flow to itself, not only by raising the general blood pressure, but by a local vaso-dilatation. I showed that double reciprocal innervation holds also in these reflexes (1902, 3, p. 292, and 1908, 2, pp. 351-353); since it appears that the dilators to the ear are probably antidromic in nature (see Bayliss, 1906, 3, p. 330), we have evidence of another case of reflex stimulation of dorsal root sensory fibres.

The usual *effect of strychnine* in converting inhibition into excitation is shown in Fig. 247 to apply to the vasomotor centres. In this figure, the effect of gradually increasing doses in converting the depressor fall into a rise is seen. In the further analysis of the action of this drug I met with inexplicable results, until I realised that it must convert, not only the inhibition of the constrictors into an excitation in depressor reflexes, but also that of the dilator centre in pressor reflexes. Thus the vaso-dilators may be *excited* in a pressor reflex after strychnine, and one obtains conversion of constriction by inhibition of dilator tone into dilatation by excitation of the dilator centre, as shown in Figs. 248 and 249.

The opposite *effect of chloroform* is well shown in Mathison's curve (Fig. 128, page 428, and in Fig. 129).

The question of the nature of the automatic *tone in the vasomotor centres* has been touched upon above (page 546).

It is well known that a considerable rise of arterial pressure occurs in asphyxia, and we have referred to Mathison's results on the action of asphyxial products in an earlier page (page 632). The experiments of Sollmann and Pilcher (1911) may be added. They find that the asphyxial stimulation of the bulbar vaso-motor centre is due to carbon dioxide, and is absent if accumulation of this acid is prevented, although oxygen is absent. We may regard it as possible that the normal stimulation of the centre is brought about by the carbon dioxide tension of the blood, like that of the respiratory centre.

Chemical Regulation of the Blood Flow.—The appropriate sensibility of the blood vessels to the products of the activity of cells, by which an automatic vaso-dilatation is caused, has been referred to in speaking of the coronary circulation (page 680). The importance of the action of metabolites in this respect was first clearly realised by Gaskell (1880, pp. 66-70). As acid products, and especially carbon dioxide, are the usual results of cell metabolism, it is natural to look for direct evidence of the effect of increase of hydrogen ion concentration. Gaskell showed that lactic acid produces decrease of tone, and I showed later (1901, 1) that carbon dioxide has the same effect. The work of Hooker (1911-1912) confirmed these results, and was extended to the action of other substances. It should also be mentioned that Severini (1876-1881) had already described dilatation of capillaries by carbon dioxide and constriction by oxygen. The fixed acid products come chiefly into play in deficiency of oxygen supply, as in asphyxia, or when oxygen is consumed at a rapid rate in great activity of the cells. Hooker showed that oxygen and also calcium ions increase vascular tone, and that carbon dioxide, urea, sodium, and potassium ions decrease it. Schwarz and Lemberger (1911) found that the injection of 1 cub. cm. of 0·001 molar hydrochloric acid into the central end of the left subclavian artery caused obvious dilatation of the vessels of the submaxillary gland, although, of course, only a part of the acid reached the gland. Comparing the action of different acids, their action was not found to correspond to the H ion concentration. But, as the authors point out, and as we have seen above (page 200), the effect is really produced by carbon dioxide driven off from the bicarbonates of the blood, which would naturally be proportional to the total molar amount of the acid introduced. Acids weaker than carbon dioxide, such as glycine and alanine, were inactive.

This local effect, it will be noticed, is opposite to that on the centres, on which, as we have seen above, carbon dioxide has an exciting influence, and Mathison showed (1911) that the direct action of potassium salts on the centres is also excitatory.

There is often present, in extracts of tissues, especially when prepared by boiling, some substance or substances which have a powerful dilator effect on blood vessels. This is the case with acid extracts of the mucous membrane of the small intestine. Now Barger and Dale (1911) have shown that such boiled acid extracts contain the salt of a base, which is also obtained by the splitting off of carbon dioxide from histidine, and is β-iminazolyl-ethylamine. The depressor substance is contained in the scrapings of the deeper layers of the mucous membrane, and can be extracted by alcohol (Bayliss and Starling, 1902, 1,

p. 335), but whether it is present in the living cells, or is given off in their normal metabolism, is not known. It is interesting to note that the vaso-dilatation, seen in the cat and dog, is replaced by vaso-constriction in the rabbit and guinea-pig, so that it would be rash to assert that this substance plays the part of a general metabolite to bring about vascular dilatation in active organs.

In contradistinction to the usual dilator action of the products of tissue metabolism, the secretion of the suprarenal bodies contains an extremely potent substance, *adrenaline*, which causes vaso-constriction in all cases where there is a supply of sympathetic vaso-constrictor nerves. This "hormone" will come up for further discussion in the next chapter. Reference is made to it here since, in many cases in which reflex rise of blood pressure occurs, there is a turning out of adrenaline into the blood vessels, which assists in the rise of pressure. The secretory nerves to the suprarenals are excited simultaneously (see especially the work of Anrep, 1912, 1).

It might occur to the reader that, perhaps, this production of adrenaline is the cause of all rise of blood pressure in pressor reflexes; if so, the existence of a vaso-constrictor centre would be superfluous. Hoskins and Wheelon (1914), however, find that four to six hours after tying off both suprarenal bodies, although there is weakness of the skeletal muscle and the heart, the blood pressure is not lowered, and pressor reflexes from a sensory nerve are still to be obtained. Thus, excitation of vaso-constrictors occurs in the absence of stimulation of secretion of adrenaline.

Similarly, the vaso-dilator nerves have been supposed by some, Barcroft (1914, p. 148), for example, to be of comparatively small importance, even if their existence is not doubtful. The products of metabolism are supposed sufficient to account for the functional dilatation. But when vaso-dilators are excited through the depressor nerve in order to relieve the heart by fall of blood pressure, it would seem a remarkable way of bringing it about if it were necessary to set a multitude of organs into activity. It must be admitted, however, that the vaso-dilators do not appear to play a great part in the reflex fall of blood pressure, since inhibition of constrictor tone is usually sufficiently effective. The decision of the question obviously rests on the proof that vaso-dilatation can occur on stimulation of a nerve apart from increase of metabolism. The effect of the chorda tympani on the atropinised submaxillary gland, in which vaso-dilatation is obtained without visible secretion, has been brought forward in evidence, but Barcroft (1914, p. 147) rightly points out that there may be stages of cell activity, preliminary to extrusion of secretion, which are not paralysed by atropine. In fact, he obtains increase of oxygen consumption. The data given seem to me to show that, although metabolites are a partial cause, there is a nervous effect in addition, since the degree of dilatation is not in proportion to the increase of oxygen consumption. Thus a 109 per cent. increase in oxygen consumption coincides with a 488 per cent. increase in blood flow, while a 50 per cent. increase coincides with an increase in blood flow of 812 per cent., that is, a larger dilatation with a smaller consumption of oxygen. Some recent experiments by Anrep and Evans (unpublished) show that it is possible to obtain vaso-dilatation in the tongue, on stimulation of the peripheral end of the lingual nerve, without any increase in oxygen consumption. In some cases an increase was found, but this was probably due to secretory activity of the glands in the tongue.

Anrep's experiments, referred to on page 349 above, show that when the secretin used to stimulate the pancreas is free from the depressor substance, there is little or no sign of vaso-dilatation in the gland, associated with secretion. The products of metabolism of active glands have not, therefore, universally a vaso-dilator action. See also Asher (1910, 2).

It was mentioned above (page 345) that the cervical sympathetic in the cat gives abundant secretion of saliva. Disregard of the fact that metabolites cause dilatation led some observers to state that the cervical sympathetic contains vaso-dilators. Now the drug, ergotoxine, obtained by Dale (1906) from ergot, paralyses sympathetic constrictors and secretory fibres, but does not affect vaso-dilators. After ergotoxine, stimulation of the cervical sympathetic fails to

produce dilatation in the gland vessels. The conclusion seems justified that the failure is on account of the absence of secretion. Some observers appear to have obtained, normally, vaso-dilatation from the cervical sympathetic, not preceded by constriction. This has not been my experience. I find constriction at first, followed by dilatation, as the secretion becomes more copious.

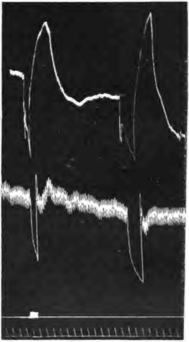

Fig. 250. Local reaction of blood vessels to fall of internal pressure.

Upper curve, volume of hind leg of dog, constrictors and dilators cut.

Lower curve, pressure in femoral artery. Zero at level of upper signal.

Time in ten-second intervals.

The abdominal aorta was compressed twice, the first time only being marked by the signal. The blood pressure curve shows the actual duration of the fall of pressure in the limb vessels in both cases.

The first fall in volume, due to deprivation of blood, is followed by a large dilatation. The magnitude of this dilatation does not seem to have any relation to the duration of the anæmia, and it is difficult to understand how an anæmia of only eight seconds duration in a curarised animal under artificial respiration could produce sufficient asphyxial metabolites to cause so large an effect.

The dilatation is followed by a constriction, which may possibly be a contractile response to the sudden inrush of blood into the dilated vessels.

(Bayliss, 1902, 2, Fig. 7.)

Reaction to Changes of Pressure.—I had observed (1902, 2), that if the general blood pressure is suddenly raised while a plethysmographic tracing is being taken of an organ, that the first passive expansion of the organ is followed by a considerable contraction. Since it was known that smooth muscle in various situations responds to stretching by contraction, I interpreted the effect as being due to a similar reaction on the part of the muscular wall of the arterioles. The fact that stimulation of the splanchnic nerves, or asphyxia, excites the flow of adrenaline into the blood current was not known at the time, and Anrep (1912, 2) has shown that the methods used by me to cause a rise of blood pressure were such as to cause this flow, and that the results are sufficiently accounted for by it. Of course, the reaction to stretching may also take place, but my experiments did not prove it. Anrep was unable to confirm the result which I had obtained by raising the pressure inside an excised piece of artery, but my recollection of this experiment is so clear that I am unable to believe that the particular piece of artery used then did not really contract. If I understand Anrep's description of his method correctly, it does not seem possible for the artery to contract when it was distended by pushing in the piston of a syringe. In the same paper I also described the opposite reaction of blood vessels to fall of pressure, in which it appeared that dilatation took place in response to diminution of tension. The effect of adrenaline is excluded here, and Anrep explains the result as due to asphyxial metabolic products. I do not feel quite satisfied with this explanation, but I was unfortunately unable to see Anrep's experiments. No doubt the prolonged stoppage of circulation in his experiments was sufficient to afford such metabolites, but if Fig. 250 be consulted, it will be seen that a compression of the abdominal aorta for eight seconds produces a subsequent dilatation of nearly as great a degree as one of twenty seconds, and it is difficult to believe that deprivation of blood flow for

eight seconds should cause an appreciable accumulation of metabolites in a *resting*, curarised leg. It is to be remembered that the blood was fully oxygenated. It is desirable that compressions of still shorter duration should be tested. If, however, we accept a reaction of the muscle cells of the arterial wall to fall of pressure, it is necessary to suppose that they must previously have been in a state of contractile response to the normal high pressure. Further, if the electrical change described by Carl Tigerstedt (page 687 above) be accepted as due to arterial contraction, it shows that the larger arteries respond to the heart beat by a contraction, although small.

Kesson (1913) was unable to find any reaction in isolated arteries, but Gesell states (1916) that stretching is the normal stimulus to the tone of the auricular muscle.

Regulation of Supply to Organs.—Summing up the facts of the previous pages, we may say that the blood flow through an organ in activity is increased in the following ways:—

1. By rise of general arterial pressure, produced by constriction in other parts.

2. By vascular dilatation in the organ itself. These two effects are combined in the Lovén reflexes.

3. By the production of acid metabolites by cell activity.

The natural tonus in arterioles is maintained in three, or perhaps four, ways:—

1. The natural property of smooth muscle to be in a state of partial tonus.

2. The continuous vaso-constrictor impulses sent out by the tonic excitation of the vaso-constrictor centre.

3. The contraction set up by adrenaline in those arterioles supplied with sympathetic nerves when this substance is present in the blood.

(4. The contraction by which they respond to the normal stretching force of the blood pressure, possibly.)

The Capillary Circulation

Satisfactory evidence regarding active changes in the calibre of the capillaries, independent of the passive effects of the blood pressure in the arterioles, and especially as to whether the nervous system exercises any control over them, has only recently been brought forward.

There appears to have been a difficulty felt owing to the structure of the capillary wall as a single layer of protoplasmic cells and the absence of a muscular coat. But we know that cells other than muscle cells can change their form under stimulation. Pigment cells of the skin in fish, frogs, and invertebrates may be mentioned, and the spherical forms taken by *Amœba* and leucocytes are familiar. The sympathetic nerve supply of the melanophores of *Fundulus* has been referred to above (page 429), and a supply of nerves to the capillaries has been described. Schafer (1912, p. 346) states that gold impregnation of the rabbit's mesentery shows every capillary to be supplied with a nerve fibre running along it, the separate nerves forming loops.

Examination of the web of the frog's foot will impress the observer with two things, amongst others: he will note how great is the volume of the capillaries in

proportion to that of the arterioles, and how much greater accordingly is the rate of flow in the latter. He will also appreciate how large a proportion of the total blood is contained in the capillary region of the vascular system, and how a small increase in the diameter of these vessels, if it occurs in a large part of the body, would suck up, as it were, a large fraction of the whole blood. The results of such a capillary dilatation will be clear from the remarks on the volume of the blood on page 689. Although the capillaries are wider, the current will be decreased, not only by the width of the stream, but also by the lowered blood pressure, which causes the flow. Under ordinary circumstances, as described by Lister (1858), Langley (1911), and Krogh (1919), the whole of the capillaries of a particular region are not filled with blood; some of them are empty and apparently contracted up. Krogh saw that in resting muscle only a small number are filled with blood. In activity, a greater or less proportion of the remainder become dilated and convey a current of blood. But a comparatively high pressure is needed to open up the collapsed capillaries by passive distension. Hence, if an effective increase of blood supply is wanted, there must be an active dilatation of the capillaries, as well as of the arterioles. The difference between the effect of arterial and of capillary dilatation is indicated by the action of cold on the skin. The colour of the skin in white races is almost entirely due to the blood in the capillaries. When these are empty of blood, the skin is white and cold. This happens in extreme cold as a result of arterial and capillary constriction. There are, however, two familiar effects of cold in which the skin is of deeper colour than normal, and, therefore, the capillaries contain more blood. In one of these, the normal healthy response, the skin is red and warm. This must be due to an arterial dilatation allowing a more copious flow of warm blood, which may be combined with capillary dilatation. The skin is thus protected from the injurious action of cold, frost-bite, and so on. The fact that the blood remains red shows that it is rapidly renewed before losing much of its oxygen. In the other state, which is more pathological, the skin is blue and cold. The blueness shows that the current must be so slow that most of the oxygen is consumed by the tissues before the blood is replaced by a fresh supply. That it is slow in transit is also shown by the coldness of the skin. Although the capillaries are widened, the simultaneous arterial constriction only allows a scanty current to pass into them.

There is also experimental evidence that the capillaries can be affected independently of the arterioles. Roy and Graham Brown (1880) noticed that the diameter of individual capillaries is not in proportion to the arterial pressure. Thus, two capillaries lying side by side may require very different external pressure to obliterate them; while, after a time, that one which previously had collapsed with the lower pressure may now require the higher one.

More complete proof of the independent contractility and dilatation of the capillaries was given by Dale and Richards (1918). The base β-iminazolyl-ethylamine or histamine had been found by Dale and Laidlaw (1910) to have the property of causing contraction of all kinds of smooth muscle, including that of the arterioles. But, when injected into the circulation of the dog, cat, or monkey, it produced the anomalous result of a *fall* in blood pressure, although its action on the arterioles should have caused a rise. By a number of ingenious experiments, Dale and Richards were able to show that a generalised dilatation of the capillaries, together with constriction of arterioles or absence of effect on them, according to the dose, is produced. In the first place, plethysmographic experiments showed a remarkable variability in the degree of expansion of a limb in relation to a given fall of blood pressure, just as would be expected from a conflict between arterial constriction and capillary dilatation in varying proportions. Next, it was shown that when a purely arterial system, obtained by cutting the mesentery at its attachment to the intestine, was perfused artificially, histamine caused a reduction of the flow by constriction of arterioles. Interesting results were obtained on the toe-pads of the cat. If the nerves of one leg are cut in a normal cat, the pads of the denervated side, although the increased volume pulse showed arterial dilatation to persist for some weeks, are *paler* than the normal side,

but at the same time warmer. This can only mean that the capillaries are less filled, but that a rapid current of blood is flowing. This was actually shown by the fact that the denervated paw warmed water more rapidly than did the normal one. The capillaries in the normal flushed paw must be wide, although its temperature shows that the arterioles are narrower. The contrast is similar to that between the "blue" and the "red" effects of cold on the human skin. When histamine was given, the denervated paw became redder by capillary dilatation. The normal paw showed first a slight decrease in colour, due to the general fall in blood pressure; later, a slight flush. The effect of a pure arterial dilator, such as acetyl-choline, is very different. The denervated paw shows no definite change of colour, because the arterioles are already dilated. The normal side becomes redder, the capillaries becoming more filled up with blood from the dilated arterioles.

The fact that adrenaline in very small doses causes a fall of blood pressure was referred to above. Dale and Richards showed that, in such cases, the action is a dilator one on the capillaries, very similar to that of histamine, except that the concurrent arterial constriction is more pronounced. This effect appears to be something independent of the typical sympathomimetic action of the drug, to be described in the next chapter. In doses larger than the minimal ones, a constriction of the capillaries is produced.

Krogh (1920) finds, in the frog's tongue, that urethane applied locally causes dilatation of the capillaries without affecting the arterioles. He shows also that weak local mechanical stimulation causes relaxation of the capillaries, and that then the venous pressure is sufficient to fill them from that side, whereas, when they are tonically contracted, the arterial pressure itself is unable to open them to any appreciable extent. Thus, their state of filling does not depend upon the arterial pressure, but upon their own degree of tonus.

Further observations showed that the innervation is antidromic, like that of the arterial dilators, as described above. The reaction to local stimulation is abolished by cocaine. It is not affected by mere section of the nerves, but disappears when these nerves are allowed time to degenerate. Thus, it is a local axon-reflex. Electrical stimulation of the lingual nerve had no effect on the capillaries, whereas strong mechanical stimulation caused marked dilatation, both of capillaries and arterioles. In my own experiments on the dorsal roots of the dog, I noticed that mechanical stimulation was very effective. According to Krogh, capillary tonus is not abolished by section or even degeneration of the nerves. But it disappears when the blood supply is cut off. It seems that this effect is not due to absence of oxygen, because it is still present if the frog is kept in an oxygen atmosphere.

The observations of Doi (unpublished) on antidromic stimulation in the frog were mentioned above. He finds that an effect is still present after acetyl-choline or after histamine. Hence he concludes that the effect is due to both arterial and capillary dilatation.

The manner in which the histamine fall of blood pressure is produced is not quite clear. I find it difficult to believe that the peripheral resistance is appreciably decreased by dilatation in a wide bed on the far side of the place of chief resistance, the arterioles. It seems more likely to be a capacity effect. Dale and Richards, however, state that the heart output is increased by small doses of histamine, although diminished by large ones. The matter requires further investigation.

Wound-Shock and Traumatic Toxæmia.—During the late war, serious trouble was caused by the state of "shock" into which wounded men fell. They showed the signs of great loss of blood, although the actual hæmorrhage may not have been at all severe. Evidence of various kinds pointed to the probability that the injured tissues were producing some toxic substance (see especially Quenu, 1919). Cannon and myself conjointly (Bayliss and Cannon, 1919; Cannon, 1919; Bayliss, 1918) found that a state similar to that of wound-shock could be produced in cats by injury to the thigh muscles, even when the possibility of nervous reflexes was excluded. Further, it was shown by Dale and Laidlaw (1919) that

histamine in particular doses had a like effect. The conclusion seems justified that there is some common factor. Although the toxic products from injured tissue have not been actually identified, they have the same physiological action as histamine; that is, a widespread dilatation of the capillaries, leading to a pooling of blood and its withdrawal from effective circulation, with all the serious results of a failure of blood supply to vital organs. A decrease in effective blood volume had been found in wounded men (N. M. Keith, 1919). If the state has not been allowed to proceed too far, the defective volume can be satisfactorily made up by transfusion of blood or even of gum saline (Keith, 1919, p. 40; Bayliss, 1918, 1920, 1). If, however, it is very severe or has lasted long, a further property of tissue-toxins, in common with histamine, combines in producing a loss of blood volume. The normal impermeability of the vessel walls to proteins is lost. In consequence of this, the osmotic pressure of these or other colloids, such as gum arabic, is ineffective, fluid leaves the vascular system, and any injected fluid, even blood, fails to keep up the volume of the blood. No treatment has been found to be of avail in such cases.

It is important to note that experience has shown that the maintenance of a normal blood volume is of the greatest moment. Dilution of the blood, up to very wide limits, is of comparatively little significance, so long as its volume is normal (see Medical Research Committee, Special Report, No. 25, pp. 26, 27).

The Veins

Since the diameter of the large veins is great, a moderately small change in their calibre might have an important effect on the total capacity of the vascular system.

It has been held by some that the veins are almost indefinitely distensible, so that they would be capable of accommodating large volumes of blood without perceptible increase of internal pressure. But, although their walls are thin, experimental evidence indicates that they are not very readily stretched passively. Roy (1881) found that the veins are less distensible than the arteries, in proportion to the changes of pressure to which they are subjected in the organism.

That veins are contractile is shown by their rhythmic contractions in the bat's wing, and by the familiar fact that stimulation causes an exposed vein to contract up. Gunn and Chavasse (1913), further, showed that adrenaline makes excised veins to contract. Thus, a sympathetic innervation is suggested. Donegan (unpublished) has made a series of experiments in which it was found that stimulation of the abdominal sympathetic brought about contraction of the veins of the leg. Thus, certain observations by Thompson and by Bancroft, in which stimulation of the sciatic nerve was found to cause contraction of the saphenous vein, were confirmed and the origin of the fibres made out.

An old observation by Goltz (1864) is of interest. When the intestines of the frog are repeatedly tapped with the handle of a scalpel ("Klopf-versuch"), in addition to inhibition of the heart, there is a maximal dilatation of the abdominal vessels, especially of the veins. If the spinal cord is now destroyed, the condition is not recovered from. Goltz concludes that the veins as well as the arteries receive a tonic innervation from the central nervous system. Tawaststjerna (1916, p. 49) obtained tracings and showed that there is a prolonged fall of blood pressure in such experiments after the vagus nerves have been cut, and that there is a great decrease in the output of the heart, showing a removal of blood from effective circulation.

The Coagulation of the Blood

The fact that the blood, when it leaves the blood vessels and comes into contact with the tissues or external objects, sets into a kind of jelly is familiar to all.

The value of this process to the organism appears to be to lessen loss of blood when blood vessels are injured.

It is impossible to give an account here of the great mass of work that has been done on the process. In point of fact, it cannot be said that it is yet understood. Much of the research done has led to little more than the multiplication of names given to supposed substances held to take part in it, but these have not been isolated as chemical individuals, and the names really refer to aspects of phenomena (see the remarks on pages 107 and 328 above).

As an illustration, I would refer to the paper by Collingwood and MacMahon (1912), where we find the following names used as applying to definite substances: fibrinogen, prothrombin, prothrombokinase, anti-thrombokinase, thrombokinase, anti-thrombin, and anti-prothrombin. These are supposed to be present before clotting. After clotting we have fibrin, thrombin, thrombokinase, anti-thrombin ?, anti-prothrombin, anti-thrombokinase, and prothrombin.

On the whole it appears that the point of view originally taken by Wooldridge (1887-1893) and developed by Nolf (1906-1908) has the most evidence in its favour. According to this theory, the phenomenon is essentially an interaction of colloids under the influence of electrolytes, especially calcium salts. There is reason to suppose that the so-called "fibrin-ferment" is not an enzyme, although some enzymes of a proteoclastic nature may be concerned in the later liquefaction of the clot.

In invertebrates there are two kinds of processes, one associated with breaking up of amœboid corpuscles, the other with a coagulation effect in the plasma. The papers by Hardy (1892) and by Tait (1910 and 1918) may be consulted.

The existence of something which prevents or retards the process of coagulation has been referred to in speaking of the extract of the heads of leeches (page 360). A similar "anti-thrombin" can be obtained from the liver, as shown especially by Doyon (1912).

On account of the difficulty and expense of procuring hirudin, it would seem worth while to attempt to prepare an anti-thrombin from the liver by Doyon's method. It would require removal of the toxic impurities present in the crude mixtures hitherto obtained. The substances in question seem to make the colloidal system more stable, so that the coagulation process induced by rough surfaces is prevented.

Zak (1912) shows that the "lipoids" of the plasma play a considerable part in the phenomena of coagulation, a fact which points to the intervention of surface action. This investigator shows that the hypothesis of a "thrombokinase" is superfluous.

SUMMARY

The object of the circulation of a fluid through the larger organisms is to supply food, especially oxygen, to the tissues, and to bring about effective interchange of chemical products.

The function of the heart as a pump to drive blood into the arteries was shown by Leonardo da Vinci, but the actual fact of the movement of the blood in a circle back to the heart was first demonstrated by Harvey. The passage of the blood through the peripheral capillaries from arteries to veins was first seen by Leeuwenhoek.

A high arterial pressure is necessary, and was shown to exist by Stephen Hales. This is in order to ensure a sufficiently rapid flow through the fine branching tubes of the various organs.

In the higher vertebrates there are two pumps in series, having the lungs between them; one is to drive the aerated blood from the lungs to the organs in general; the other to drive the venous blood, returning from these organs, through the lungs, in order that it may take up oxygen and lose carbon dioxide. The two pumps are combined in one organ, the heart, but their cavities are separate.

The greater part of the work done by the heart muscle is expended in raising the pressure of the blood driven out, and may be measured by the product of the volume and pressure.

Determination of the time course of the pressure curve in the ventricle shows that no blood is expelled until the maximum tension is developed. The muscle, therefore, works at its best efficiency. During the expulsion of blood into the aorta, the pressure in the ventricle remains nearly constant, the curve showing a flattened top.

The inflow from the veins is, practically, the determining factor in the work done by the heart. The human heart, indeed, can deal with as much as 21 litres per minute. Thus it is the *length* of the fibres that determines the energy given out.

A brief account is given of the heart sounds.

The oxygen consumed by the heart is in direct proportion to the energy of the tension developed, and is the same at 15° as at 36°. The actual amount of oxygen used depends on whether the "reserve-stuff" of the heart itself is oxidised, or the glucose of the solution perfused; but the relation between the energy produced by oxidation and that of the tension developed is constant. The effect of excess of carbon dioxide is to prevent the conversion of chemical to mechanical energy; similarly, the presence of calcium is necessary for the due conversion of this chemical energy into that of tension.

The muscular tissue of the vertebrate heart initiates the beat, and is also responsible for the transmission of excitation from one part of the heart to another. In the mammal, a localised bundle, that of His, conveys the excitation from auricles to ventricles. The rate of the automatic rhythm is greatest in the sinus tissue, so that this acts as the pace-maker. In the mammal, a remnant of sinus tissue, the "Keith-Flack" or "sino-auricular" node, is the initiator of the beats, and is in direct connection with the nerves controlling the rate of the heart beat.

The heart requires an abundant supply of oxygen, which is provided by the copious flow of blood through the coronary circulation. The arterioles of this system are very sensitive to the dilating action of products of the muscular metabolism.

The inhibitory action of the vagus nerves may show itself in different ways, on rate, strength, conducting power, or excitability of the muscle. These effects appear to depend chiefly on the particular function of the tissue in which the fibres end. The duration of the state of excitation is lessened by vagus stimulation, a fact which explains the abolition of the T-wave of the electrocardiogram by the vagus, since its action is naturally more pronounced at the base.

There are also excitatory nerves, the accelerators, supplied to the heart muscle; their action is directly opposed to that of the vagus nerves.

Both kinds of nerves can be excited reflexly.

The importance of the elastic nature of the walls of the arteries is pointed out. It accommodates, temporarily, the blood driven out by the rhythmic beats of the heart, converting the flow through the capillaries into a continuous one. Incidentally, it gives rise to the pulse wave. This wave is due to the elastic recoil of the arterial wall, and must not be confused with the actual mass movement of the current of blood.

The resistance to flow in the blood vessels is due to the internal friction of the blood, so that changes in the viscosity of the blood change the resistance.

The total volume of blood in an animal is a function of the outer surface of the animal.

A brief description is given of the methods used for the investigation of changes in the heart and circulation.

The arterioles are supplied with two kinds of vasomotor nerves, vaso-constrictor or excitatory, and vaso-dilator or inhibitory, in respect of the normal tonus of the arterial muscular wall.

The constrictor fibres are all of sympathetic origin. That of the dilators is more varied. In some organs it is peculiar, the vaso-dilator impulses being conveyed by the ordinary sensory fibres in an "antidromic" direction. These fibres to blood vessels are, apparently, lateral branches of the sensory fibres, and can thus give rise to axon reflexes, as in inflammation.

While stimulation of sensory nerves in general causes rise of blood pressure by reflex arterial constriction, there is one set of nerve fibres, arising from the aorta and the heart, which always produces reflex fall of blood pressure. These are known as the fibres of the depressor nerve.

In reflex rise of blood pressure, excitation of the vaso-constrictor centre is combined with inhibition of the tone of the vaso-dilator centre. In reflex fall, excitation of the vaso-dilator centre is combined with inhibition of tone in the vaso-constrictor centre. Reciprocal innervation holds, therefore, as in the case of skeletal muscle.

Stimulation of the central end of the afferent nerve from an organ causes reflex dilatation in the organ itself, with constriction elsewhere, thus ensuring the maximal supply of blood to the organ.

Strychnine and chloroform show their usual actions of converting inhibition to excitation, or excitation to inhibition, respectively, on the vasomotor reflexes. Owing to the complex nature of these reflexes effects are sometimes produced of a nature difficult at first to analyse.

The effect of products of metabolism of active organs is to cause dilatation of the blood vessels, thus ensuring an automatic regulation of blood supply. But the facts do not account for the whole of the vaso-dilator phenomena met with, which require the existence of vaso-dilator or inhibitory nerves.

The reflex secretion of adrenaline is not necessary as an accompaniment of pressor reflexes, so that we require also the existence of a vaso-constrictor centre.

There is some evidence that the muscular wall of the arterioles responds to stretching by a contraction, but the question is not yet definitely decided.

The capillaries are capable of active changes in calibre independent of those of the arterioles. Their innervation is of the dilator antidromic type. They have an inherent tonus, not dependent on the central nervous system. Certain drugs act on capillaries only, while others act in an opposite manner on arterioles and capillaries.

The veins are not particularly distensible. They have a tonus, apparently dependent on sympathetic innervation. Stimulation of the sympathetic supply causes contraction.

The coagulation of the blood is an interaction between certain colloidal systems under the influence of electrolytes, chiefly calcium salts. The intervention of surface action is shown by the accelerating effect of rough surfaces and by the action of lipoids.

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CHAPTER XXIV

HORMONES, DRUGS, AND TOXINS

THAT there are a number of poisonous substances which act on living organisms in very minute doses has been known for centuries, but the fact that there are also many chemical compounds which are indispensable to the normal activities of organisms, although present in infinitesimal amount, has been recognised, in its full import, only in recent years. We have seen instances in the "accessory factors" of diet (pages 254-261), in the chemical mechanism of secretion, and of vascular dilatation and constriction, as well as in enzymes and catalysts in general. In most of these cases the active substance is present in such extraordinarily small amount that, at present, it seems almost impossible to discover its nature. In some few cases the chemical nature is known.

The Minuteness of the Quantity necessary is made clear by the work of Bertrand on the action of zinc and manganese (pages 221-222 above), and that the phenomenon is not peculiar to living protoplasm is shown by the experiments of Elissafov on the action of thorium on the sign of the electric charge on surfaces. Thus the presence of 0·2 mg. of thorium nitrate in a litre of water lowered the rate of movement through a quartz capillary, under the action of electric forces, by 50 per cent. Other cases will appear presently. The nature itself of catalytic action, indeed, implies that only a very minute amount of a catalyst is necessary in order that an effect may be produced.

HORMONES

When we came across the mode by which the pancreas is excited to activity, it became obvious, to Starling and myself, that the chemical agent concerned is a member of a class of substances of which others were previously known. The peculiarity of these substances is that they are produced in one organ, and carried by the blood current to another organ, on which their effect is manifested.

Since some confusion has been introduced into the nomenclature of the subject, a few words are necessary as to the history of the name.

The group of substances referred to, which includes adrenaline and the various internal secretions, is characterised by the property of serving as chemical *messengers*, by which the activity of certain organs is co-ordinated with that of others. They enable a chemical correlation of the functions of the organism to be brought about through the blood, side by side with that which is the function of the nervous system (see the Croonian Lecture by Bayliss and Starling, 1904). This being so, it seemed desirable and convenient to possess a name to distinguish the group. That of "internal secretions," already in use, did not sufficiently emphasise their nature as messengers. After the discovery of secretin, this name for the group was for a long time a subject of discussion in the laboratory, but no satisfactory name was suggested. Finally, Mr W. B. Hardy proposed the name "hormone," derived from ὁρμάω ("I arouse to activity"), and, although the property of messenger was not suggested by it, it was adopted. It has, in fact, been generally understood as having the meaning intended, and not to be applied to any kind of substance which excites activity. Indeed, a name of such very wide application would be of comparatively little value. I may give three quotations to show that this property of messenger is usually understood in the use of the word "hormone." Gley (1911, p. 19, footnote) points out that the "excitants fonctionnels (*hormones* de Bayliss et Starling)" are of two kinds. We shall presently return to this distinction, but the point is that Gley insists on the correlation established between different organs "par l'intermédiaire de substances sécrétées par des glandes spéciales et déversées dans le sang qui les transporte là où elles peuvent agir" (p. 21). Again, Hustin (1912, p. 319) says—"Bayliss et Starling donnèrent le nom *d'hormones* (ὁρμάω, j'excite) à ces substances qui constituaint, comme la *sécrétine*, des intermédiaires chimiques entre des organes

voisins ou situés à distance." Babkin (1914, p. 5) says—"Die Hormone bilden die Vermittler zwischen den verschiedenen Teilen des Körpers (Bayliss und Starling)." When, therefore, the name is extended to apply to such substances as chloroform or toluene, which set into activity enzymic changes in cells because they are able to penetrate the cell membrane and enable interaction to take place between constituents within the cell (H. E. and E. F. Armstrong, 1910, 1911), it appears to me that the original meaning of the word is deprived of significance and applied to cases of a different kind, for which there does not seem to exist the necessity for a special name.

It may be added that this conception of co-ordination by chemical messengers is to be found in a note by Brown-Séquard and d'Arsonval (1891), who say—"Nous admettons que chaque tissu et plus généralement chaque cellule de l'organisme sécrète pour son propre compte des produits ou des ferments spéciaux qui sont versés dans le sang et qui viennent influencer par l'intermédiaire de ce liquide toutes les autres cellules rendues ainsi solidaires les unes des autres, par un mécanisme autre que le système nerveux."

When we look around the numerous examples of such influence of minute traces of substances formed by one organ and acting on other organs, we note that there are not many so definite as that of secretin, where the food entering the duodenum causes the production of a special substance which enters the blood and excites the pancreas to pour into the duodenum a digestive juice, and, so far as we know, does not act on any other organ except the liver, whose secretion is an adjuvant to that of the pancreas.

Gley (1911, p. 19) rightly calls attention to the fact that some of the substances which act like hormones in modifying the activity of distant organs, such as carbon dioxide on the respiratory centre, are really products of the ordinary metabolism of cells, and are not, like secretin, produced for a specific purpose. The delicate sensibility of a particular nerve centre to carbon dioxide must be supposed to be an adaptation developed in the course of evolution. Gley suggests calling these latter substances "*parahormones*." He also points out the convenience of a name for that class of hormones which influence growth, and proposes that of *harmosones* (from ἁρμόζω, I regulate or direct).

When distinction is required between the different classes of hormones, these names appear satisfactory. On the other hand, the distinction made by Schäfer (1913) between substances which excite (hormones) and those which depress (chalones) activity, seems unnecessary, as also the name "autacoid" to include both. If we interpret, as we are justified in doing, "excitation to activity" as being equivalent to "bringing into play an influence on cell processes," this influence may be of such a nature as to inhibit. Moreover, such a typical hormone as adrenaline excites blood vessels to contraction, but inhibits the muscular coat of the intestine, so that it is both hormone and chalone, according to the particular way in which the sympathetic end organ, on which it acts, terminates in the cell.

The name "internal secretions" has been given to many of the substances with which we are here concerned; this was done before the discovery of secretin. The fact that many organs deliver the products of their activity into the blood current was well known to Claude Bernard (1859, ii. pp. 411, 412), and the name "internal secretion" is due to him. The products of organs such as the suprarenals, the thyroid, and so on, are those to which the name is given.

Before we pass on to consider some facts in relation to various individual hormones, the considerations of Hopkins, to which attention was directed above (page 20), should be remembered. An intermediate product in a chain of reactions, although its concentration in the system at any given moment may be infinitesimal, is probably of great importance as a necessary stage. The amount present may be small because the rate of the reaction producing it may be slow, compared with that of the reaction by which it is changed into a further product.

Individual Hormones

Secretin.—As already pointed out, the most typical of all the chemical messengers is that which causes secretion of pancreatic juice when acid enters the duodenum. This mechanism was described in a previous chapter (pages 344 and 346).

The view of Popielski that the effect on the pancreas is merely due to the presence of a vaso-dilator substance is easily disproved in many ways. Bayliss

and Starling (1902, 1, pp. 336, 337) showed that the fall of blood pressure could be prevented by extraction of the mucous membrane with absolute alcohol before boiling with acid, and that acid extracts of desquamated epithelial cells of the intestine had no action in depressing the blood pressure, although very active on the pancreatic secretion. Recently Launoy and Oeschlin (1913) have confirmed this conclusion in a completely convincing manner. Although the substance acting on the pancreas is soluble in 90 per cent. alcohol, it is insoluble in absolute alcohol. Thus, if concentrated aqueous solutions of secretin, prepared in the usual way by the action of hydrochloric acid on the duodenal mucous membrane, are poured into excess of absolute alcohol, a precipitate is obtained. This process, several times repeated, results in the production of a white powder, easily soluble in water, insoluble in absolute alcohol. As Fig. 251 shows, it has a powerful secretory action but no depressor action on the blood pressure. On the other hand, the alcoholic mother-liquors, concentrated, give a powder of yellowish colour, also soluble in water, which has a powerful effect on the blood pressure, together with a very small one on the secretion, no doubt due to small amounts of secretin left unprecipitated. From the work of Dale and Laidlaw (1910), it seems more than probable that the fall of blood pressure is due to β-iminazolylethylamine.

As yet we have no definite knowledge of the chemical nature of secretin. It is evidently an intensely powerful substance, but does not appear to have a very complex structure, since it is diffusible through parchment paper. It is, naturally, incapable of acting as an antigen, since the production of an anti-body in the blood would be antagonistic to its function. The statement also applies to other hormones. It was suggested by Bayliss and Starling that secretin is produced by the action of acid on a precursor in the cells of the mucous membrane. To this supposed precursor the name "prosecretin" was given. A certain amount of discussion has since taken place as to whether secretin itself is not present in the cells. The work of Stepp (1912) shows that it may occasionally be present in small quantities, so that mere extraction with boiling water is sometimes sufficient to obtain solutions of active secretin, as was indeed found by Bayliss and Starling (1902, 1, p. 340). In most cases no such effect was obtained. In certain of these cases it was found that the slightly alkaline opalescent solution, obtained by boiling and filtering, contained a substance from which, by boiling with acid, an active secretin was obtained. These results indicate that the cells usually contain a precursor, but it is not surprising to find that it should sometimes happen that the secretin produced by action of acid, etc., on these cells has not completely passed away into the blood stream. Stepp also comes to the conclusion that, however prepared, secretin is one and the same substance. He gives a method by which a permanent dry preparation can be obtained, which is similar to that of Launoy and Oeschlin, but giving a better yield by the use of ether to precipitate.

The method of preparation of very active solutions, worked out by Dale and Laidlaw (1912, 1), depends on the fact that mercuric chloride precipitates secretin as a mercury compound, soluble in dilute acids, insoluble in neutral or weakly alkaline reaction; it may, however, merely be held in adsorption by a substance having these properties. In this method, a large amount of impurity is stopped at the outset. It was easy to obtain a preparation of which 1 c.c. produced 8·5 c.c. of juice.

We may next consider briefly some results obtained by Lalou (1912, 1). Bayliss and Starling noted the fact that solutions of secretin introduced into the lumen of the gut failed to excite the pancreas. Hence the agents causing the production of secretin, when they are introduced into this cavity, must act directly on the cells and, at the same time, enable the secretin to pass into the blood vessels. Lalou calls attention to the fact that various agents, such as saccharose, urea, etc., produce secretin by action on the mucous membrane *in vitro*, but do not excite pancreatic secretion in the living animal. It has not been shown as yet, however, that such agents, which destroy the cells *in vitro*, really produce secretin in them in the living state, so that it seems to me that the

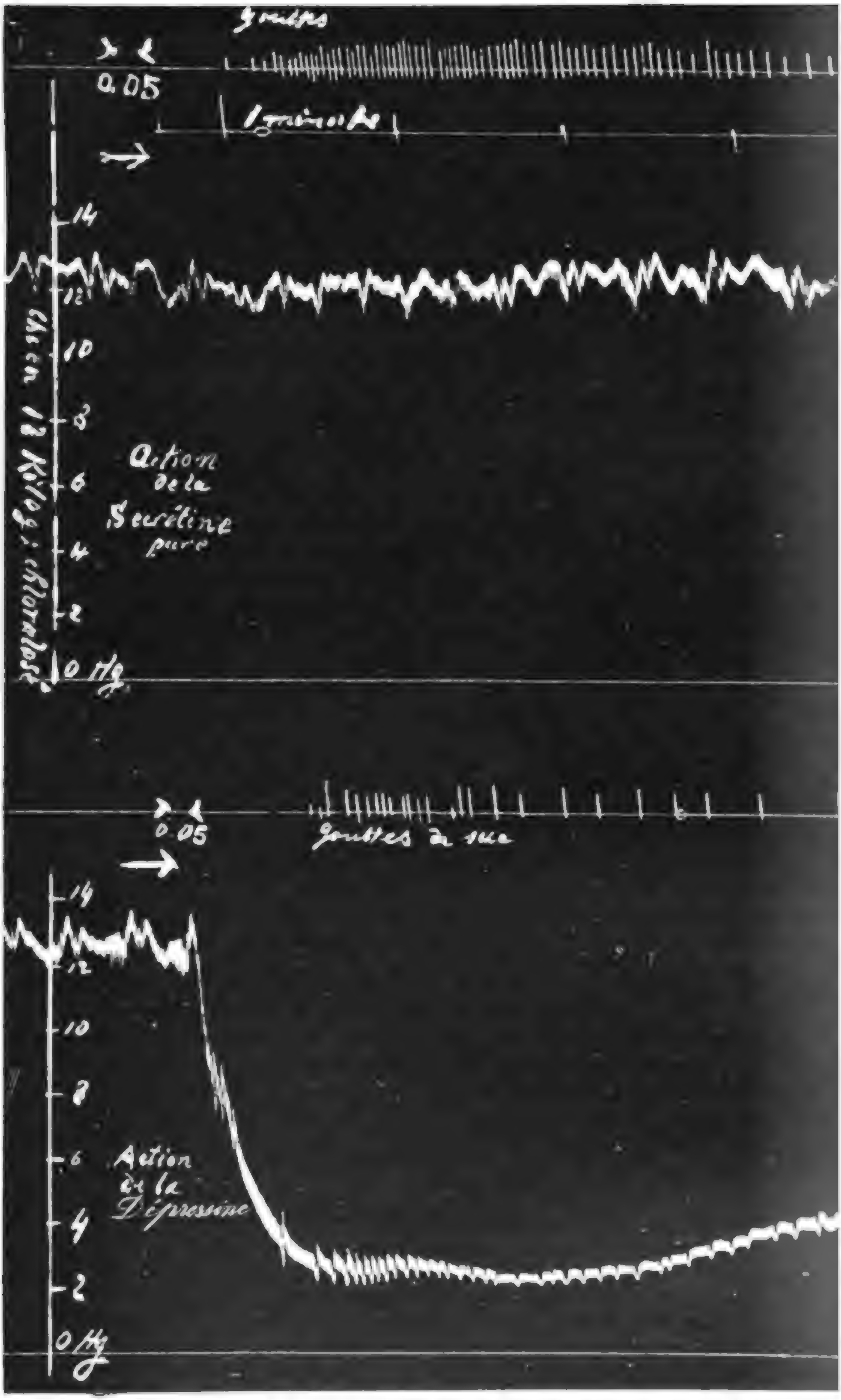


FIG. 251. ACTION OF SECRETIN ON THE FLOW OF PANCREATIC JUICE.

In both tracings, the top line registers the drops of juice falling from a canula in the pancreatic duct.

The curve below it shows the arterial pressure, with scale in centimetres at the side.

In the upper tracing, time is shown in minutes below the drop recorder.

The upper tracing shows the effect of secretin from which the depressor substance had been removed by extraction with alcohol in the manner described in the text (page 708). There is no fall in blood pressure but a vigorous flow of juice.

The lower tracing shows the effect of the depressor substance extracted from the raw secretin. It still contained a certain amount of the active secretin. There is a large and prolonged fall of blood pressure, probably due to β-iminazolylethylamine, but only a small secretory effect.

(Launoy et Oechslin, 1913.)

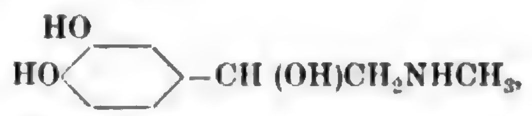
distinction drawn between excitants such as hydrochloric acid, which enable secretin to be absorbed, and those above named, which produce it, but without enabling it to be absorbed, is not a valid one. In a further paper (1912, 2) Lalou investigates the behaviour of secretin towards chemical agents, with a view to elucidating its nature. The most interesting fact is that it is rapidly destroyed by pancreatic and gastric juices, by erepsin in neutral solution, and by papain. In connection with this fact, a discovery by Delezenne and Pozerski (1912) is of interest. They showed that extracts of various tissues containing erepsin, especially the mucous membrane of the intestine, have a powerfully destructive action on secretin. This fact has to be kept in mind when extracting the mucous membrane with cold water.

Gley (1912) gives a classification of the various chemical excitants of pancreatic secretion.

The complete proof that secretin is present in the blood of an animal after the introduction of acid into the duodenum was given simultaneously by Fleig (1903) and by Enriquez and Hallion (1903), who found that the blood of a dog, in which pancreatic secretion had been induced by the introduction of acid into the duodenum, was capable of producing activity of the pancreas in a second dog.

Gastric Secretion.—The observations of Pavlov showed that the introduction of meat into the stomach caused secretion in an isolated small stomach, even when nervous influences were excluded. Edkins (1907) showed that extracts of the pyloric mucous membrane, made in various ways, but especially by the action of dextrine, caused increased formation of an acid gastric juice. Maydell (1913) confirmed the fact, in so far as that subcutaneous injection of extracts of pyloric mucous membrane brought about increased secretion in a dog with a chronic gastric fistula. The most active preparation was found to be made by extracting pyloric mucous membrane with 0·4 per cent. hydrochloric acid at ordinary temperature. Immediately before injection this was neutralised. Extracts of other parts of the stomach or of the duodenum were ineffective. Comparing the properties of the juice obtained from the same animals by "sham feeding," that is, the juice obtained by natural stimulation of the vagus, the acidity was found to be about the same; the digestive power was, however, considerably less in secretion obtained by chemical agency. Maydell was able to obtain a dry active preparation by the application of Stepp's method, described above for the pancreatic secretin.

Adrenaline.—Brief reference has been made already to the action of the product of activity of the suprarenal glands. The first investigation of the properties of this substance was made by Oliver and Schäfer (1895), in so far as concerns extracts of the organs. They showed also that the pressor substance is contained in the medulla only. The active principle, "adrenaline," was isolated by Takamine (1901) and found to be



and may be regarded as a methyl-amino derivative of pyrocatechol. Since this contains an asymmetrical carbon atom, there are two optical isomers. In the suprarenal glands the *l*-form only occurs. The racemic mixture has been prepared synthetically by Stolz (1907), and found to be rather more than half as active as the natural form; hence the *d*-isomer is much less active than the *l*-isomer. An instructive case of similar difference of activity in optical isomers will be referred to in the case of hyoscine on a later page.

The similarity of the structure of adrenaline to that of tyrosine or homogentisic acid (see page 432 above) will be noticed, and it has been stated that the suprarenal gland mixed with tyrosine *in vitro* is able to produce adrenaline from the amino-acid; but further investigation failed to confirm the statement.

The occurrence of adrenaline in the secretion of the "parotoid" glands of a toad, described by Abel and Macht (1913), indicates that it may be a more or less accidental product of metabolism in its first appearance.

It is an intensely active substance. Pysemsky and Kravkov (1912), in perfusing the ear of the rabbit with Ringer's solution, to which adrenaline was

added, found that one part in two hundred and fifty millions of the saline solution could be detected.

The cells which secrete adrenaline, that is, those of the medulla of the glands, stain a brownish-yellow colour with potassium bichromate; hence the name given to them of "Chromaffine" tissue. Similar cells are found throughout the vertebrate kingdom in various situations. In the lamprey the system is arranged segmentally. The reaction is also given by certain nerve cells in invertebrates, and J. F. Gaskell (1914 and 1919) points out that the presence of such cells is correlated with the development of a contractile vascular system. In the leech, each segmental ganglion contains six chromaffine nerve cells, and the contractile vascular system consists of a series of segmental units, each under the control of a segmental ganglion. The chromaffine cells contain a substance similar to adrenaline, and the contractile vessels react to adrenaline as those of the vertebrate do. There appears then to be some close connection between these chromaffine nerve cells and those of the medulla of the suprarenal bodies; moreover, the relation between the action of adrenaline and the sympathetic system, already spoken of, shows a further connection. J. F. Gaskell suggests that the chromaffine nerve cells of the invertebrate are the common ancestors of the adrenaline-secreting chromaffine system and the sympathetic nervous system of the vertebrate. Thus we find the contractile vascular system regulated both by the sympathetic nerves and by secretion of adrenaline.

Further evidence is found in the mode of development of the medulla of the suprarenals. As Balfour showed (1878, pp. 242-245), this has the same origin as the sympathetic system, and Kohn (1902 and 1903) showed that the development of these cells of the medulla is from a series of groups of cells in connection with the sympathetic along the body axis. Rudiments remain for some years in scattered situations, as along the aorta and to form the carotid gland, but the main mass becomes the suprarenal ganglion, or medulla of the suprarenal body. The scattered remains are called *paraganglia*.

We have seen that one of the characteristics of the sympathetic outflow is the connection of each fibre with a cell before passing on to its destination. Now Elliott (1913, 2) has shown that the supply to the adult suprarenal gland has no cell station previous to the cells of the medulla themselves, a further fact in evidence of the similarity of these cells to those of the sympathetic ganglia. Elliott (1913, 1) points out that there are two types of cells to which the sympathetic fibres from the spinal cord pass:—

(1) The sympathetic ganglion cell, which is distally united to the plain muscle cell by its axon process, and so provides a path for the nervous impulse.

(2) The medullary or paraganglion cell, which is not in connection with the muscle, but is equally innervated from the spinal cord, and secretes a chemical substance into the blood, which can produce an identical stimulation of the muscle through its myo-neural junction.

These may have been originally identical, and the liberation of adrenaline an essential part of the nerve impulse. But, at the present time, the paraganglion cell secretes adrenaline, which maintains the smooth muscle in a state of excitability, ready to react to the nerve impulses from the sympathetic fibres. Elliott's general scheme is reproduced in Fig. 252.

According to Elliott (1914) the vaso-constrictor fibres of the splanchnic nerve lose their excitability after removal of the suprarenals. It would appear, therefore, that the presence of adrenaline is, in some way, necessary to the activity of the vaso-constrictor mechanism. It will be remembered that all the nerves of this kind are of sympathetic origin.

Fascinating as this scheme is, there are some minor points which are not completely cleared up. The sweat glands, although innervated by the sympathetic, are not excited by adrenaline. Further, we have seen that the presence of the suprarenal bodies is not necessary for the production of vascular constriction. It may well be, however, in this latter case, that the loss of excitability does not take place rapidly.

The dilator action of adrenaline on the capillaries seems to be an effect independent of its relation to the sympathetic.

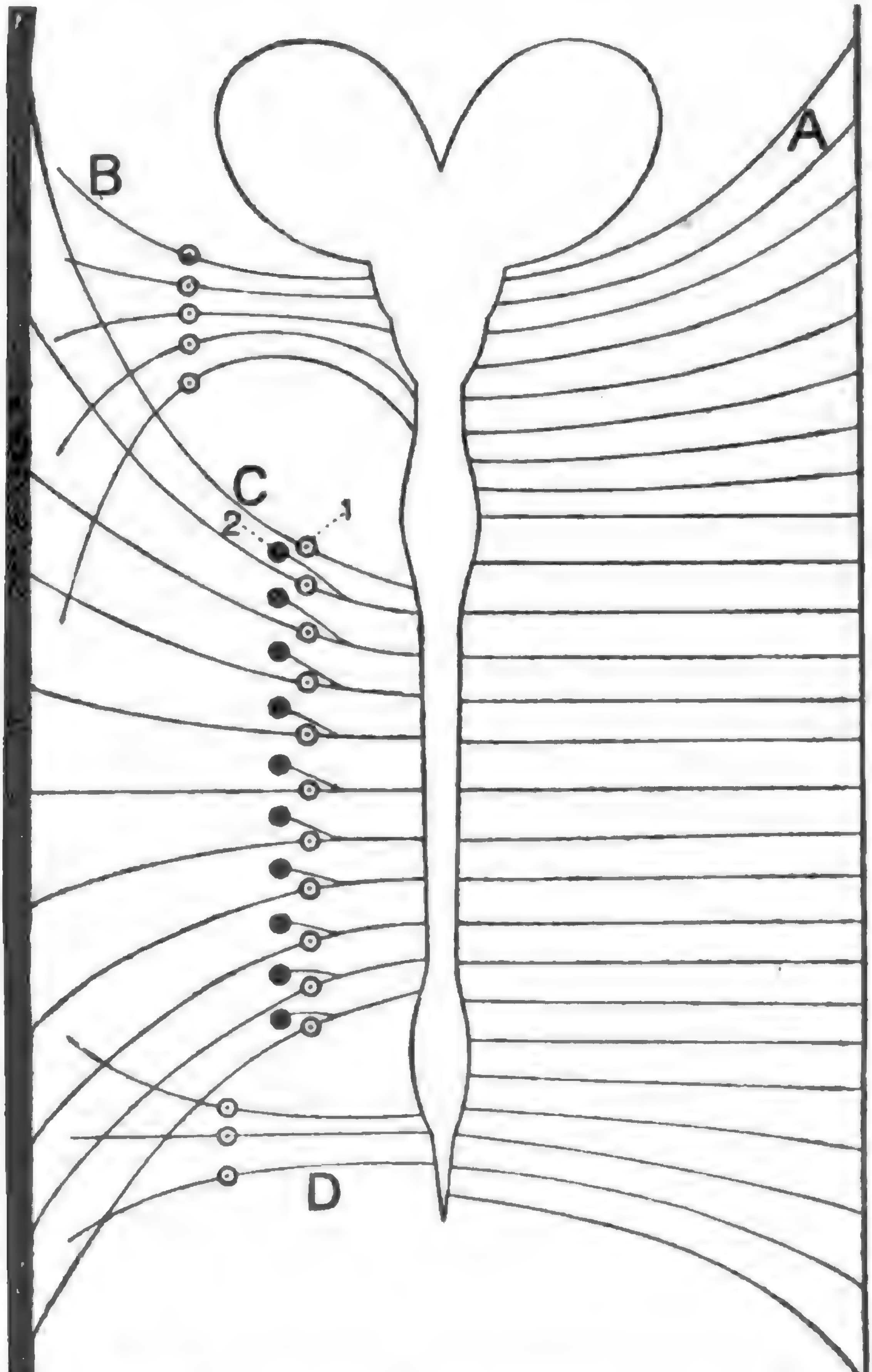


FIG. 252. ELLIOTT'S DIAGRAM OF THE EFFERENT NERVES FROM THE CENTRAL NERVOUS SYSTEM IN THE MAMMAL.

A, The non-ganglionated ordinary motor nerves to striped muscle, which are distributed segmentally only. On the left side these are omitted for simplicity, and only the autonomic or ganglionated visceral nerves to plain muscle are indicated.

Of these, *B* is the cranio-cervical outflow in the vagus, etc.; *C*, the thoracico-lumbar or sympathetic proper; *D*, the sacral outflow, or pelvic visceral nerve to the bladder and colon. All these subdivisions contain both excitatory and inhibitory nerves.

C_1 is the sympathetic ganglion cell; C_2 the paraganglion cell, secreting adrenaline, the chief mass of these being concentrated to form the medulla of the adrenal gland, though a few, even in adult life, may be found elsewhere in relation to the various sympathetic ganglia. The black rectangle innervated by the nerves from the cells C_1 represents the mass of plain muscle which is also stimulated by adrenaline, that is, by the secretion of C_2.

Afferent sensory nerves and their posterior root ganglia are all omitted from the diagram. Their course from the viscera is not clearly known.

(Elliott, 1913, 1, p. 313. "From Brain.")

Elliott (1912) has shown that the various states associated with stimulation of the splanchnic nerves cause discharge of adrenaline into the blood, causing rise of blood pressure, and the other results of sympathetic stimulation. Such states are fright, anæsthesia, stimulation of afferent nerves, and so on. Reference to the work of other observers will be found in the paper. Those of Cheboksarov (1910) and Cannon (1915) may be especially mentioned.

Suppose, then, that a drug is given which stimulates the splanchnic nerves. It is clear that the effects obtained will be combinations of those of the drug itself with those of the adrenaline sent into the blood. Dale and Laidlaw (1912, 2) have found that nicotine and pilocarpine produce effects of sympathetic stimulation on the cat's uterus *in situ*, but not when excised. Also the effect of nicotine in causing dilatation of the pupil, after the sympathetic supply had been cut off, was found to be absent if the suprarenal bodies were excluded from the circulation. The glycosuria produced by puncture of the floor of the fourth ventricle is probably also due to secretion of adrenaline.

Although, under experimental conditions, there seems to be no doubt that the blood of the suprarenal vein contains more adrenaline than that of the artery, some discussion has arisen as to whether the normal blood pressure is, under normal conditions, maintained to any extent by a constant inflow of adrenaline. When the effect of the venous blood from the suprarenal gland on the arteries of the frog is compared with that of known concentrations of pure adrenaline, and this again with the amount required to produce a permanent rise in the blood pressure of the mammal, it appears that the amount sent into the blood by the unstimulated suprarenals is too small to produce any perceptible result. Further, Trendelenburg (1914) was unable to find any difference between the average blood pressure in cats, unanæsthetised and quiet, before and directly after removal of the suprarenals.

Adrenaline is, then, a hormone, used only for special purposes, and unlike some of those to be mentioned presently, which are in constant activity.

The Cortex of the Suprarenals.—Elliott (1913, i. p. 316) points out the remarkable fact, although it does not appear to have any physiological significance, that so many ductless glands, the pituitary, suprarenals, thyroid, pancreas, testis, etc., are of double nature. This renders analysis difficult.

The cortex of the suprarenals has no particular relation to the sympathetic nerves. The presence of a considerable amount of a lipoid substance appears to be an indication of a healthy state of activity. It is supposed that the absence of the cortex is associated with the bronzing of the skin in Addison's disease. There is also evidence that overgrowth of the cortex in children is associated with sexual precocity and premature adolescence.

Carbon Dioxide.—The distinction of this substance as a *parahormone* by Gley, and the development of special sensibility on the part of the respiratory centre to increase of hydrogen ion concentration in the blood, caused by its presence, have been already referred to. The work of Hasselbalch and Lundsgaard (1911), and of Hasselbalch (1912), may be added as containing the most accurate determinations of the hydrogen ion concentration of the blood in connection with stimulation of the respiratory centre.

It seems very doubtful whether carbon dioxide has any particular function as a hormone in any other respect. The "acapnia" of Mosso, as responsible or mountain sickness, has been shown by Haldane and his co-workers not to be the correct explanation. Yandell Henderson has published a series of papers in the *American Journal of Physiology*, from 1908 onwards, advocating the importance of carbon dioxide as a necessary constituent of the blood, and explaining various phenomena as being due to its too small concentration. In so far as its removal reduces the optimal hydrogen ion concentration for numerous processes, this removal has, of course, an injurious effect. The evidence that other apparent effects cannot equally well be explained in other ways is not very strong.

The Reproductive Organs.—It has been known for centuries that removal of the sexual glands produces profound changes in the organism. But it is only comparatively recently that exact observations have been made on the phenomena.

Perhaps the most striking results to commence our brief study with are those

of Steinach (1910). If the testes are removed from frogs, the "clasp reflex" is abolished. Of course, the nerve centres concerned remain, and it is not surprising that, after some time, indications of the reflex may return. The absence of it might depend, however, on the absence of afferent nervous impulses from the testes. Steinach, then, took a number of castrated frogs and tested for several consecutive days whether the reflex could be evoked. Having found that it could not, he injected, into the dorsal lymph sac, the substance of testes of frogs which had shown a marked reflex. After about twelve to twenty-four hours the reflex began to appear, reached a maximum in two days, and disappeared in three to four days, but could be brought back by renewed injections. No increased excitability in any other reflex could be detected, and it was noticed that the peripheral receptors, the swellings on the thumbs, were enlarged after injection of testicular material. The effect of the testis of the same species is the greatest, but it is not strictly specific, since that of *Rana fusca* will act on *R. esculenta*. The result was still more marked in cases, about 4 to 8 per cent. of the frogs caught, where the reflex was naturally absent. Steinach believes that the action is exercised, primarily, on the central nervous system, since injections of nervous matter from normal males caused the return of the reflex in castrated males; while the central nervous system of castrated males had no such effect. The testes of males for two or three months after the breeding season were devoid of action, so that the hormone is formed periodically. It is supposed to act by depressing the activity of centres which inhibit that for the clasp reflex.

Further experiments were made on rats. Finding that feeding with testis material was ineffective, autoplastic transplantation in animals of three to six weeks old was performed. The testis was removed to various positions on the inner surface of the abdominal muscles in some animals, and removed altogether in other animals. In the latter, no development of vesiculæ seminales, prostate, nor penis took place. In those in which the transplanted testis grew, the development of the organs named was indistinguishable from that of normal males, and the animals behaved, sexually, just as these. The hormone concerned did not arise from the generative cells themselves, because they were not developed in the transplanted testis, whereas the interstitial substance was fully developed.

Interesting observations have been made by Marshall and Hammond (1914) on the effect of removal of the testes in Herdwick rams, where the operation is found to stop the growth of horns. It is shown in these experiments that the theory of Geoffrey Smith, according to which the effect of the testes is not due to a hormone, but to a process explained by Ehrlich's side-chain theory of the production of antitoxins, does not hold.

Turning to the female, we find interstitial tissue in the ovary, as we saw in the testis, to which the development of sexual characters is apparently due. The changes taking place in the first stages of pregnancy have been shown by various observers to depend upon the development of the corpora lutea, which are formed in the place of the Graafian follicles after the ova have been extruded. The reader may be interested to examine Figs. 253 and 254, which are copied from Réné de Graaf's drawings of the follicles, which he discovered, and of the corpora lutea. The effect of the latter on the development of the mammary glands will be considered in the next section. In this place we may refer to the work of Ancel and Bouin (1910), who showed that the growth of the uterus is dependent upon that of the corpora lutea, since if these latter are formed in any way, the first stages of the uterine hypertrophy occur, although there may be no pregnancy. In this latter case the uterus returns to its original state. If the Graafian follicles are ruptured artificially, it is found that the uterine hypertrophy occurs, but only when a corpus luteum is formed. Further, if the corpora lutea are destroyed by the cautery when uterine hypertrophy has become obvious, the hypertrophy ceases to increase and rapidly disappears.

From the experiments of Marshall and Jolly (1908) and those of Nattrass (1910), it follows that a transplanted ovary, when it continues to live, is capable of maintaining the sexual characters of the individual.

With regard to transplantations of the ovary, Guthrie (1908) believes that

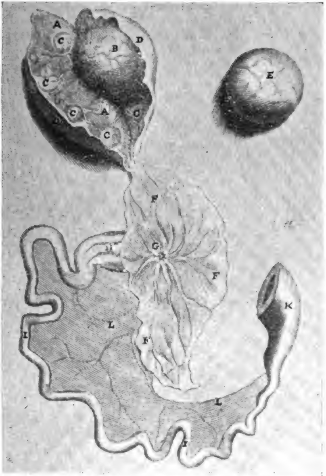


FIG. 253. REGNIER DE GRAAF'S FIGURE OF THE OVARY OF THE COW BEFORE COITUS, CONTAINING RIPE OVA IN GRAAFIAN FOLLICLES.—His description of the figure is as follows:—

"Exhibet Testiculum sive Ovarium Vaccinum apertum, prout illud ante coitum observari solet.

AA, Testiculus secundum longitudinem apertus.
B, Ovum maximum seu maturum in Testiculo adhuc contentum.
CC, Ova minora seu immatura in Testiculo hærentia.
DD, Membrana Testiculorum Dartos appellata.
E, Ovum maximum e Testiculo exemptum.
F, Tubæ Fallopianæ membranosa expansio.
G, Foramen coarctatum in Tubæ extremitate existens.
H, Tubæ Fallopianæ extremitas.
II, Tubæ pars reliqua.
K, Cornu uterini pars abscissa.
L, Tubæ Ligamentum in hominibus alis vespertilionum assimilatum."

(Regnier de Graaf, 1677, Tab. decima-quinta.)

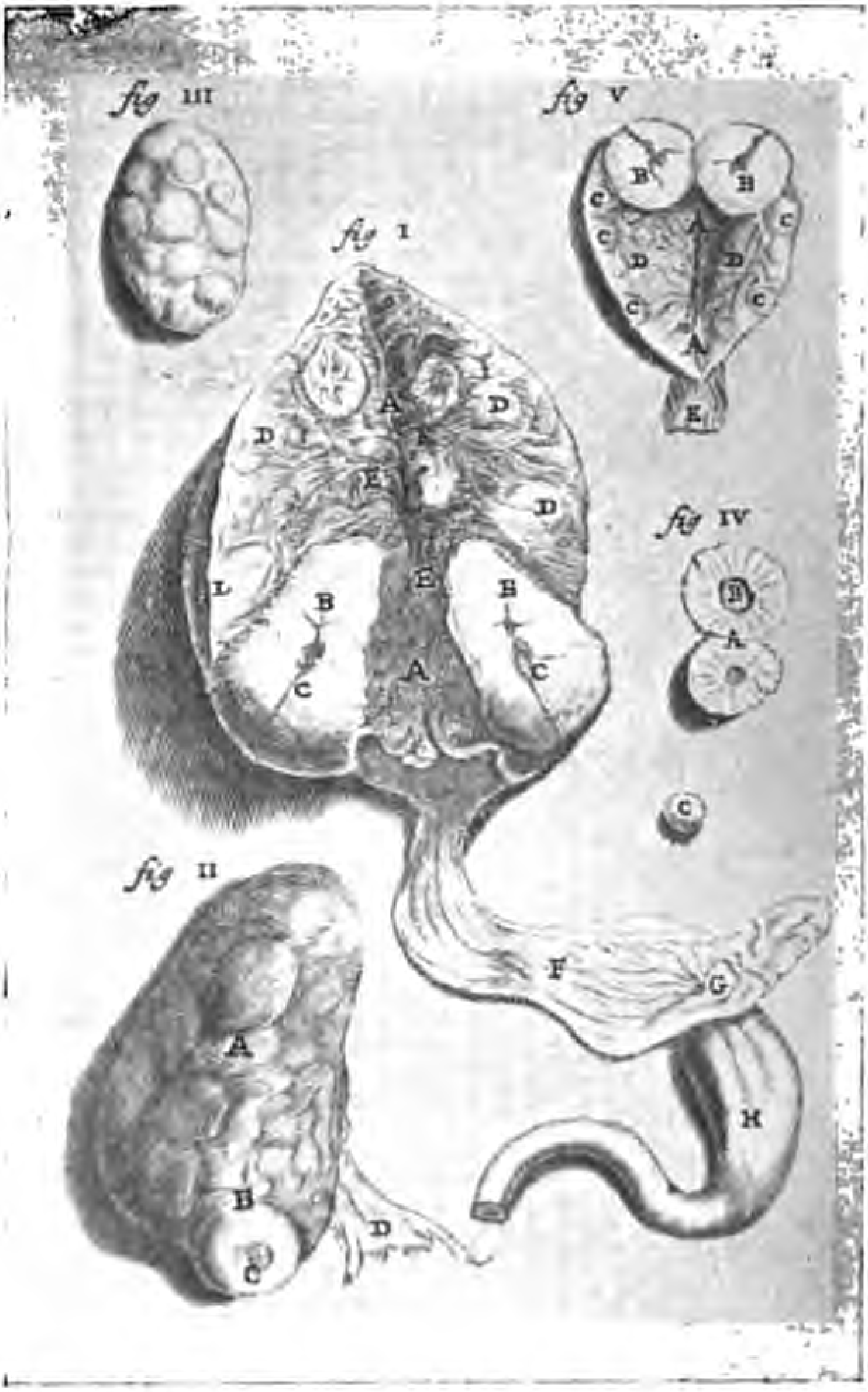


Fig. 254. Changes occurring after coitus. Formation of corpus luteum.

"Vaccæ et Ovis Ovarium exhibet, ut ea, quæ post coitum in illis eveniunt, conspiciantur.

Fig. I. Exhibet Testiculum Vaccæ.

A, Testiculus secundum longitudinem apertus; *BB*, Glandulosa substantia, quæ post Ovi expulsionem in Testibus reperitur, per medium divisa; *CC*, Cavitas, in qua Ovum contentum fuit, fere abolita; *DD*, Ova diversæ magnitudinis in Ovario contenta; *EE*, Vasa sanguinea ad Ova excurrentia; *F*, Tubæ Fallopianæ membranosa expansio complicata; *G*, Foramen in extremitate Tubarum existens; *H*, Tubæ Fallopianæ pars abscissa.

Fig. II. Exhibet Testiculum necdum apertum.

A, Testiculus; *B*, Glandulosa substantia extra Testiculum protuberans; *C*, Foramen in ejus medio existens; *D*, Tubæ Fallopianæ membranosæ expansionis portio.

Fig. III. Exhibet Testiculum Ovillum cum transparentibus Ovis necdum masculino semine irroratis.

Fig. IV. Exhibet glandulosam globulorum substantiam ex Ovis Testiculo exemptam prout Ovum adhuc continebat.

A, Glandulosa globuli substantia adaperta; *B*, Locus ex quo Ovum exemptum est; *C*, Ovum ex eo exemptum.

Fig. V. Exhibet Testiculum Ovis ex quo Ovum ab aliquot diebus expulsum fuit.

A, Testiculus per medio divisus; *B*, Glandulosa globulorum substantia cum cavitate sua propemodum abolita; *CC*, Ova diversæ magnitudinis in Testium superficie hærentia; *DD*, Vasa sanguinea ad Ova excurrentia; *E*, Ligamenti Testiculorum portio."

(Regnier de Graaf, 1677, Tab. decima-quarta.)

he has obtained evidence that the "foster-mother" has an influence on the offspring. He took hens of black and of white Leghorn varieties, which were found to breed true, when mated with cocks of their own colour. The ovaries were then interchanged. Subsequently they were mated with cocks of the colour corresponding to the transplanted ovary, but opposite to their own colour.

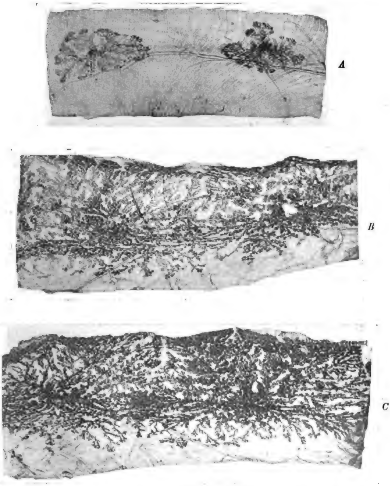


Fig. 255. Growth of mammary gland of rabbit caused by formation of corpus luteum.

A, Two glands of the virgin.
B, Two glands, five days after coitus not resulting in fertilisation.
C, Two glands of virgin rabbit, four days eighteen hours after appearance of corpora lutea, provoked by artificial rupture of the follicles with scissors.

(Ancel et Bouin, 1911, Figs. 1, 2, and 3.)

The majority of the offspring were found to be spotted. For instance, when the ovary and the male were pure white, the foster-mother pure black, the chickens had black spots.

Should this turn out to be correct, we see a possibility of the disputed "*transmission of acquired characters*," since the body of the mother affects the germ plasm. In respect to the question in general, the remarks of Shattock (1911, pp. 26-34) will be found of much interest. He shows that the callosities of the monkey are not to be attributed to transmission of

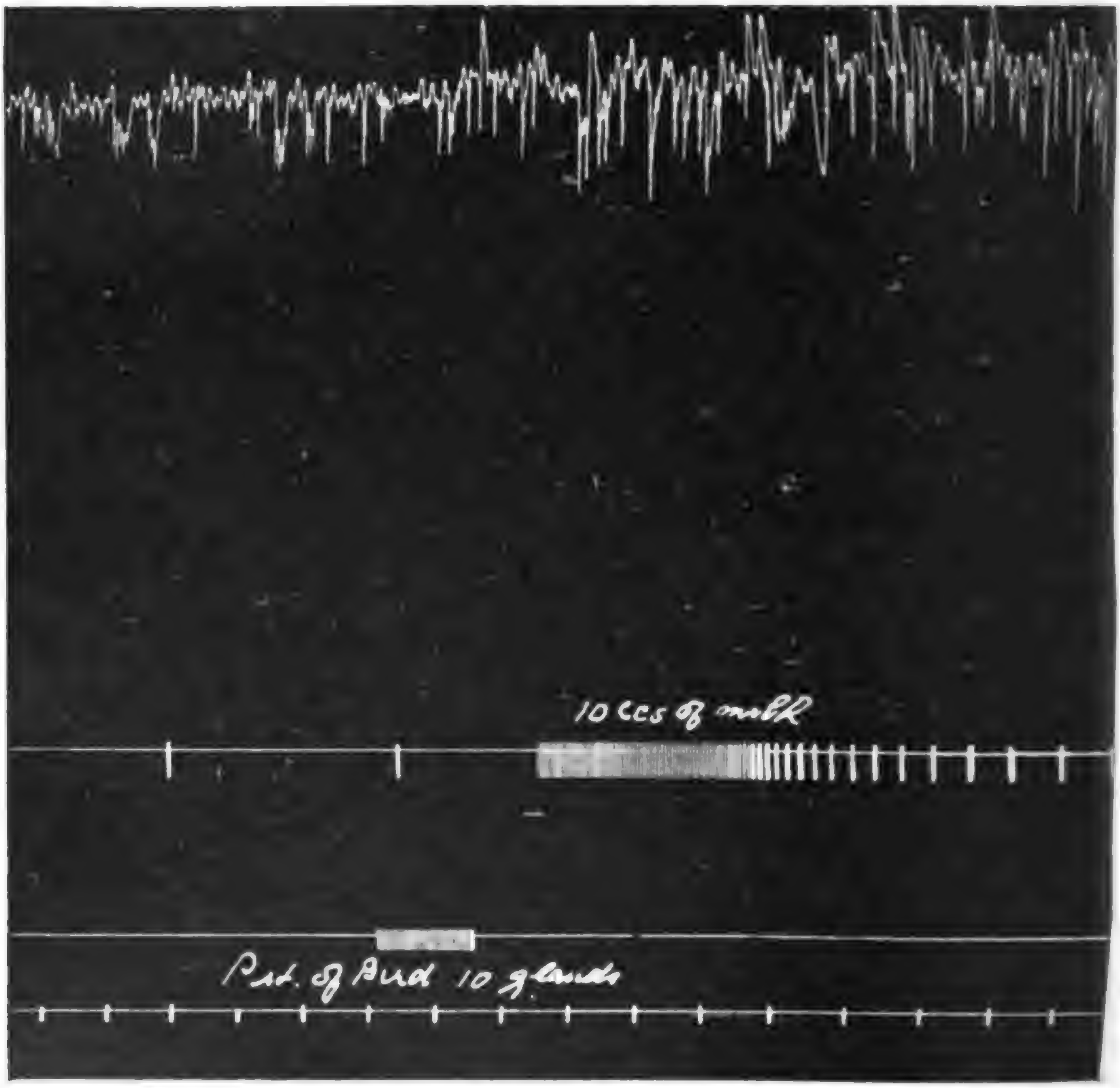


FIG. 256. SECRETION OF MILK IN THE CAT, PRODUCED BY INJECTION OF PITUITARY EXTRACT FROM THE FOWL.

Upper curve, blood pressure.
Top signal, drops of milk secreted.
Middle signal, injection of saline extract of ten pituitary glands of the fowl.
Bottom signal, time in ten-second intervals.

(Mackenzie, 1911, Fig. 4.)

a character acquired by friction. A callosity acquired in this way is not transmitted, but has to be regained after birth.

The Mammary Gland.—The growth of this organ is closely connected with that of the uterus in pregnancy, so that it is not surprising to find that the growth is affected by a hormone produced in the corpus luteum. This has been shown by O'Donoghue (1911, 1 and 2, and 1913) and by Ancel and Bouin (1911). The artificial production of corpora lutea by puncture of the Graafian follicles is followed by growth of the mammary gland, as shown in Fig. 255.

The second stage, associated with secretory activity in the later period of pregnancy, is independent of the corpus luteum. It has been shown by

Mackenzie (1911) that the gland is not under the influence of the nervous system, but that extracts of various organs, injected into the blood current of a cat in lactation, cause secretion of milk. The organs found active were the pituitary body, the corpus luteum, the pineal body, the involuting uterus, and the mammary gland itself. The pituitary body is by far the most active; the substance responsible is in the posterior lobe, and that of the bird is capable of exciting the mammary gland of the cat (see Fig. 256). The fœtus and placenta produce hormones which inhibit the gland. Further analysis of the action of pituitary extract was made by Hammond (1913). The effect is said not to be due to pressing out of milk by contraction of muscle in the ducts, since, with other evidence, after increase of secretion there is no sudden drop, followed by return to the normal rate, as would be the case if the ducts had to be refilled. The daily yield of goats was found to be only slightly increased by injections, so that pituitary extract seems to act by setting free the constituents of the milk, rather than by causing increased formation. The theory is suggested that the precursor of milk-protein and lactose (perhaps a glyco-protein) is caused to take up water, become hydrolysed, and by the increased osmotic pressure cause the inflow of water to the cells and the washing out of the fat which has accumulated at the ends of the cells. Maxwell and Rothera (1915) regard the effect as a true secretory one.

The Pituitary Body.—In addition to the effect on the mammary gland and that on the kidney (page 359), this organ has other effects, especially on growth.

The gland consists of two parts. From the posterior, nervous part the hormones above mentioned are obtained, together with one which excites plain muscle in general to contraction.

The anterior part secretes an eosinophile material, which, according to Herring (1908), passes into the third ventricle, and thus into the cerebro-spinal fluid.

Disease of the gland shows it to have a powerful influence on growth and metabolism. Cushing (1912) regards the state of acromegaly or gigantism as due to excessive activity, and that of obesity, with eunuchoid changes, as due to failure of pituitary hormone. The results of experimental interference are somewhat in dispute as yet.

The Thyroid Gland.—Here, again, analysis is difficult because of the double nature of the organ.

Absence of thyroid prevents growth, and produces the remarkable state of myxœdema, associated with cretinism. Excess of the hormone causes Graves's disease, exophthalmic goitre. In both cases nervous phenomena are met with.

Gaskell (1908, Chapter V.) brings strong evidence to show that the thyroid gland of Ammocœtes, and therefore of vertebrates generally, is derived ancestrally from the uterus of the original palæostracan. There is still a connection between the generative organs and the thyroid which is a matter of popular knowledge, and it seems not unlikely that remains of the internal secretion may have continued when its original function ceased.

The most interesting fact, chemically, with regard to the thyroid is the high content in iodine, which appears to be present in a complex organic iodine compound, united with a protein. That this is the active principle is shown by the fact that the effect of thyroid substance, which is active even when taken by the mouth, is in proportion to its iodine content (see Kendall, 1917).

The Parathyroid.—This is held by Paton, Findlay, etc. (1917), to regulate the metabolism of guanidine, since the "tetany" which results from extirpation can be imitated by injection of guanidine.

The Thymus.—Gudernatsch (1912), by feeding tadpoles on thyroid or thymus, respectively, obtained precocious metamorphosis in the first case, retardation in the second case, so that enormous tadpoles resulted. These results have been confirmed by later workers.

The thymus has thus a relation to growth. It is large in the young animal. Halnan and Marshall (1914), however, failed to detect any effect when the gland was removed from growing guinea-pigs.

The Internal Secretion of the Pancreas.—Mering and Minkovski (1889) showed that complete removal of the pancreas invariably results in severe diabetes mellitus. The animals excrete large amounts of glucose, even if no carbohydrate food is given. They show great hunger and thirst, and, in spite of liberal food, they die of inanition in the course of two or three weeks, or less. To obtain this result extirpation must be complete; one fifty-fifth of the organ suffices to prevent the symptoms. Since total extirpation is of primary importance for success, the excellent method of removal introduced by Hédon (1910) may be referred to.

The glycogen vanishes from the liver in pancreatic diabetes, and although the glucose content of the blood may be raised to 0·8 per cent., no glycogen is stored, although it has been stated that fructose may give rise to glycogen in the liver.

The meaning of this great loss of glucose is still obscure. Investigations directed towards testing the power of the tissues to consume carbohydrate have not been able to show that the power is entirely wanting (see the paper by Patterson and Starling, 1913), although it seems to be diminished, especially in the later stages. A. H. Clark (1916) found that the addition of Locke's solution which had been perfused through a normal pancreas always resulted in a greater utilisation of glucose by the diabetic heart.

Although no attempts to prevent the diabetes by the injection of extracts of pancreas have been successful, the transfusion experiments of Hédon (1913) show that the glycosuria is due to the absence of a hormone secreted by the normal pancreas. An anastomosis was made between the vein of the pancreas of a normal dog and the jugular vein of a depancreatised and diabetic dog. The glycosuria was almost abolished, and there was a diminution in the glucose content of the blood. That the liver plays an important part in the process is shown by the following variation of the experiment. A part of the normal pancreas of another dog was intercalated, by vascular anastomosis, in the circulation of a diabetic dog. This had a similar effect to the previous form of experiment, *but only* when the venous blood of the pancreas, presumably containing the hormone, was allowed to pass through the liver, by anastomosis with the splenic vein of the diabetic dog. The serum of the venous blood of the pancreas is said to have no anti-diabetic power. The fact of the relatively small effect on the glucose content of the blood leads Hédon to the view that the hyperglycæmia and the glycosuria are more or less independent. The blood sugar may be scarcely diminished at all when its excretion by the kidneys ceases owing to the influx of normal blood. But, since the blood sugar did not increase, it is clear that either the excess production had been retarded, or the rate of consumption by the tissues increased; otherwise it must accumulate when excretion stops. These experiments indicate, then, (1) that the liver plays an important part, and (2) that there is some influence exerted by the pancreatic hormone on the excretion of sugar by the kidneys. This latter may be either decreased permeability, or, perhaps more probably, an effect on the reabsorption in the tubules of the glucose contained in the glomerular filtrate. A third possibility is suggested by Hédon, namely, that there might be some change in the state in which the sugar exists in the blood.

In connection with these results, the experiments of De Meyer (1906-1910) are of interest. He finds that the liver, perfused with Ringer's solution, loses less glycogen if pancreatic extract is added. If the liver came from a depancreatised animal, it was found that its function of storing glycogen could be restored by the perfusion of fluids containing pancreatic extracts. Perfusion with blood, instead of with Ringer's solution, showed still more marked effects of addition of pancreatic extract. Perfusion of the kidney with Ringer's solution containing glucose, together with pancreatic extract, showed that considerably less sugar came out in the secretion than in the absence of pancreatic extract. De Meyer finds that the addition of such extract to solutions of glucose does not diminish its rate of diffusion through a colloidal membrane, and interprets the effect as being due to a diminution of the permeability of the kidney for glucose. It might, of course, be exerted on the power of reabsorption. The

experimenter is inclined to attribute the action, both in the case of the liver and the kidney, to the increase of hydrogen ion concentration in the blood in diabetes, which is counteracted by the internal secretion of the pancreas.

The experiments of Cohnheim, confirmed by Hall (see references in Levene and Meyer's paper, 1911), showed that, while the addition of muscle plasma or of pancreatic extract to solutions of glucose had practically no effect in causing fall in copper-reducing power, mixtures of the two had a considerable effect. The conclusion was naturally drawn that the effect of the pancreas was to facilitate the consumption of glucose by the muscles. But Levene and Meyer (1911) found that the reducing power of such sugar solutions after action of combined muscle and pancreas extracts was restored to its original height by boiling with 1 per cent. hydrochloric acid. Further, the apparent disappearance was only to be obtained with concentrated glucose solutions. If the product of the action of the combined extracts was diluted ten times and allowed to stand, the original reducing power returned. It was evident, therefore, that the effect was due to the activation of some enzyme system, which acts, as usual, in a synthetic manner on glucose, in a hydrolytic manner on the disaccharide formed in concentrated solutions of glucose. Further experiments showed that dilute solutions of maltose were hydrolysed by the mixture, whereas it was shown later (1912) that lactose was not so hydrolysed, and that synthesis occurred neither with mannose, xylose, ribose, nor galactose, but that it did with fructose. The experiments, interesting in themselves, show that the phenomenon has nothing to do with diabetes.

The next point that comes up for discussion is the origin of the hormone, since there are two different tissues in the pancreas, the cells which secrete the digestive juice and the structures called the "*islets of Langerhans*." Although it had been suggested that these latter are the organs which secrete the anti-diabetic hormone, certain observers had advocated the view that they do not constitute a tissue *sui generis*, but are produced from the ordinary alveoli of the gland. The question was finally decided by the work of Homans (1912). The results of Bensley, showing that the islets could be stained selectively, both after fixation and by intravital injection of methylene blue, neutral red, or pyronin, were first confirmed, so that it was possible to detect changes in the size or number of the islets. If the gland is excited to prolonged activity with secretin, no change in the islets can be detected. If only a small part of the gland is left in an animal, no conversion of acinous tissue to islet tissue occurs, as might be expected to happen if it were possible. Previous investigators had found that the pancreas, by injection of paraffin into the ducts and so on, could be reduced to a state in which no normal acinous tissue could be found, although the islets remained and no diabetes occurred. Homans points out, however, that the decisive proof of the connection of the islets with carbohydrate metabolism is not hereby given unless it is shown that the remains of the gland acini play no part and could be removed without diabetes occurring. At the same time, evidence distinctly points to the islets as the responsible tissue. Fig. 257 reproduces three of those given by Homans.

Interrelation of Internal Secretions.—Various statements have been made as to the mutual relation of these organs, especially by Eppinger, Falta, and Rudinger, who have based elaborate theories on very slender evidence. Elliott (1913, p. 320) justly warns against building on insecure foundations, saying, "Medicine owes no debt of gratitude to those who teach to her theories without proof."

Nevertheless, as Elliott himself (1913) points out, there are common features which suggest a common bond:—

(1) Carbohydrate metabolism is influenced, not only by the pancreas, but also by the thyroid in superactivity, in acromegaly, and by the injection of adrenaline.

(2) Growth is affected by the testis and the cortex of the suprarenals, arrested by absence of the thyroid.

(3) Nervous implications.

(4) The pituitary becomes hypertrophied when the thyroid is removed. Acromegaly may lead to enlargement of the thyroid.

(5) Gaskell (1908, p. 430), on morphological grounds, classifies together the

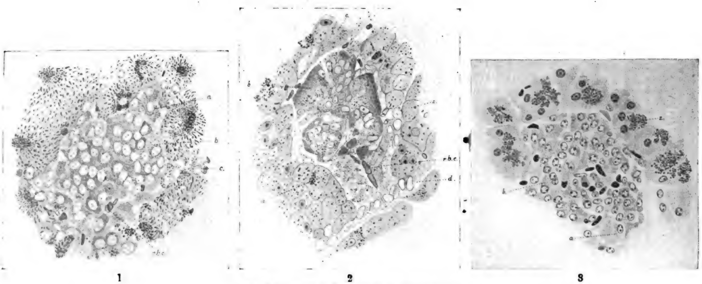


FIG. 257. ISLETS OF LANGERHANS IN THE PANCREAS.

1. Normal islet in the *charged* pancreas of the dog.
   a, *A* cells of Lane. *b*, *B* cells of Lane. *c*, Centroacinous cells. *r*, *b*, *c*, Red blood corpuscles.

Notice the arrangement of the large round zymogen granules in the surrounding acini, also the distribution of the "mitochondrial" filaments. The borders of the islet are extremely irregular, and no limiting membrane is present.

2. Normal islet in the *exhausted* pancreas of the dog.
   a, *A* cells of Lane. *b*, *B* cells of Lane. Notice the distribution of their granules along the capillaries, which are indicated by the red blood corpuscles (*r*, *b*, *c*).
   c, Centroacinous cells. *d*, Duct cells, which are continuous with those of the islet below and low down on the right. *z*, Exhausted acini.

Notice the prevailing absence of zymogen granules, the presence of some mitochondrial filaments, and the vacuolisation of the cells. In the low right-hand corner a small group of acinous cells is cut off from the rest by a small duct.

3. Normal islet in the charged pancreas of the guinea-pig.
   a, *A* cells of Lane, not differentially stained. *b*, *B* cells of Lane, containing fine blue granules. *z*, Acini, containing zymogen granules.

(Homans, 1912, Figs. 1, 2, and 3.)

suprarenal cortex, the pituitary, and the thyroid as being modified from the coxal glands, the primitive excretory organs of the ancestral arthropod (see also Cannon and Cattell, 1916).

Hormones in Plants.—Although there is no such effective way of chemical interchange in plants as there is in the circulating blood of animals, there is distinct evidence that chemical products of one part are able to influence the activities of other parts.

The lateral roots, which normally grow horizontally, can be made to grow vertically downwards if the main root is removed. Errera (1904) investigated, in pines, the corresponding change of direction of growth of a branch into a vertical stem when the apical bud of the main stem is removed. He suggested that the apical bud of the main stem forms some kind of an internal secretion, which prevents the upward growth of the lateral shoots as long as this apical bud is present. See also Loeb's work (1917) on *Bryophyllum*, and his theory of root and stem-forming hormones.

Keeble (1910, pp. 135-137) considers that such "chemical stimulators" play a part in the transfer of the activity of localised cambium cells to others in their neighbourhood. In the case of *Convoluta Roscoffensis*, the signal for the commencement of the later phases of development owes its origin to the presence of the green algal cells, without whose concurrence, probably by the production of a hormone, no kind of artificial feeding has been found to be effective.

Mention may also be made of the substance extracted by rain from grass, which has been shown by Pickering (1911, see also Russell, 1912, p. 112) to be injurious to apple trees. Grass should not be grown in orchards.

The Action of Drugs and of some other Chemical Compounds

There are some of these substances which will receive mention in the present section for two reasons. The first is that, although the subject properly belongs to pharmacology, it is clear that the mode of action of the hormones of the previous section cannot be understood until we know more of the action of drugs on cells. The second is that certain alkaloids and other active principles are of great value as means of investigation, owing to their action as excitants or paralysers of particular kinds of cells or nerve endings.

Their Mode of Action.—The preparation by Barger and Dale (1910) of a series of amines, which were found to possess the power which adrenaline has of stimulating sympathetic endings, but in different degrees, gave the opportunity of comparing this property with their chemical structure. Details of the latter will be found in Barger's monograph (1914). It was found that approximation in structure to that of adrenaline was associated with increased intensity of action, and more definite restriction to the sympathetic system. The optimum carbon-skeleton for this purpose consists of a benzene ring with a side-chain of two carbon atoms, of which the terminal one is attached to an NH_2 group. This is further intensified by the presence of two hydroxyls on the benzene ring in the 3 : 4 position relative to the side-chain. These substances are, therefore, catechol derivatives. Of these bases, those with a methyl-amino group, including adrenaline, produce the inhibitory effects of the sympathetic, such as that on the intestine, more powerfully than the excitatory effects on the blood vessels, etc. The opposite is true of the primary amines of the same series. It is to be noted, however, that a catechol nucleus is not essential. Catechol itself has no action of the kind referred to; while not only parahydroxyphenyl-ethylamine, but also iso-amylamine, are powerfully active.

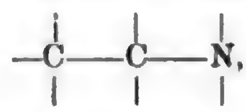
As has often been pointed out, in comparing the activity of a series of related substances it must not be forgotten that, in altering the chemical composition, we alter in many ways the physical properties also. We have to reckon with changes in solubility, in ability to pass through the cell membrane, in approximation to the colloidal state, in surface tension, in rate of diffusion, and so on.

Barger and Dale, in the paper mentioned, give a valuable discussion of the theoretic aspect of the question, from which I take the following considerations. They show that the ease of oxidation has nothing to do with activity. Since

there is evidence that the excitatory and inhibitory effects can be varied independently, reason is given for the belief that the myo-neural junctions concerned with inhibition are not identical, in their relations to chemical substances, with those concerned with excitatory effects. Ergotoxine, as we shall see presently, is of interest in this respect.

It is difficult to reconcile the view that the sympathetic nerves produce their effect by the liberation of adrenaline at the myo-neural junction with the fact that a base, which only differs from adrenaline by the absence of the methylation of the amino group, is as active. Why should it set free an allied substance, but not a more active one? The difficulty is further increased by the fact that certain inhibitory effects, as on the non-pregnant uterus of the cat, are relatively more easily produced by adrenaline than by nerve stimulation, whereas some motor effects, such as pilo-motor action, are more easily produced by nerve stimulation than by adrenaline.

The fact that these bases have very definite relations to the cells of a certain morphological system shows that there "must be something in these cells, or connected with them and them only, which has a strong affinity for these amines." But it is pointed out that this property is by no means necessarily the same as that which confers stimulant activity on the amines. As I pointed out in connection with catalytic action, the adsorption of a substance on a surface is independent of the chemical action it may exert on the material of the surface after adsorption. Barger and Dale further call attention to atropine and pilocarpine, whose localisation is practically identical, while their action is opposite. Thus also the peculiar distribution of the action of nicotine, or of the sympathomimetic amines, does not necessarily depend on the existence of specific chemical receptors in the cells peculiarly sensitive to them. It may be that in some cells the stimulant substance easily reaches the site of action. The authors further find "the theory of receptive side-chains very difficult to apply" to their results. If the relation is one of chemical union, it seems that the points of constitution common to all the active bases should give an indication of the nature of the chemical receptor in the cells which combines with them. But there is only one common complex, namely:—



and this "exists in innumerable bases with no sympathomimetic activity." That physical factors intervene is indicated by the fact that differences in the relative activity of pairs of substances appear in the course of an experiment, and occasionally in an individual cat as compared with other cats. On the purely chemical view, it would be necessary to assume that there is a different chemoreceptor for each amine, and that these may vary independently of each other; a view very difficult to maintain, since "the number of possible sympathomimetic amines is indefinitely large." The conclusion arrived at is that "the least unsatisfactory view seems to us to be that which regards the existence of stimulant activity as dependent on the possession of some chemical property, the distribution and, in the main, the intensity of activity as due to a physical property."

The remarks of Straub (1912, p. 4) are also of interest. "The theory of the selective distribution of active substances in the organism has, as a necessary foundation, a purely material taking up of them by the cell. What happens in the cell in presence of the substance, how it gets there, and why it is held fast is the next question. It is frequently answered (as by Ehrlich) by the statement that the substance, as a chemical individual, reacts with chemical constituents of the chosen cell, with satisfaction of affinities and formation of a chemical compound. I hold this explanation, in its general aspect, as too far-reaching and inappropriate, and, in its results, as unfruitful. There are an indefinite number of substances which have a constitution scarcely capable of reactions in the organism, such as nitrous oxide, carbon dioxide, potassium salts, and many of those substances called indifferent narcotics, on account of their passivity; one cannot imagine with what cell molecules they are to show chemical affinity, since this affinity of the cell

molecules arises merely from ordinary organic chemistry. If one is to operate with 'chemo-receptors' for all of these numerous cases, one mystery is merely changed into another. The existence of chemo-receptors for poisons is not to be denied, but this is not a universal fact, and therefore no general theory can be based upon it." There are also some definite experimental facts which show that it does not apply to certain typical cases. Straub himself (1907) has shown that the action of muscarine on the heart of Aplysia, and Neukirch (1912) that the action of pilocarpine on the mammalian intestine, are absent after the drug has entered the cells, and are only manifest when it is in the act of either entering or leaving. Further, Neukirch (p. 166) shows that no perceptible diminution in the strength of dilute solutions of pilocarpine is produced by the lying of the intestine in them, a fact difficult to understand if it were taken up in chemical combination in the cells.

Additional interesting facts come out in the investigations of Straub (1910) on strophanthin, a glucoside of the digitalis group. When injected into the organism, its action is most marked on the ventricle of the heart, next on the other heart cavities, and finally on the blood vessels. The active doses are extraordinarily small. Straub had previously found that the action of *alkaloids* is reversible, in that they can be regained from the organs which had stored them up, by simple washing with water. But on testing the case of strophanthin, he found that it could not be regained by washing out. It seemed possible that it had entered into a definite chemical compound with the heart muscle, or some constituent thereof, although it is difficult to see what kind of a compound, undecomposed by boiling, such a chemically inert substance as a glucoside could form with cell materials. On this account Straub thinks it always necessary to test other possibilities before making the assumption of a chemical union. On proceeding to investigate the question further, the unexpected result was obtained that, contrary to the case of the alkaloids, the reason why no glucoside could be extracted from the cells was because there was none there. The cells had not taken it up at all. Thus, if 1 c.c. of a solution containing 0·01 mg. of the drug, which is sufficient to produce a powerful action without killing the ventricle, be perfused backwards and forwards, it is found, by applying it to a second heart, that it is quantitatively as powerful as at first. The method of measurement was carefully worked out, and will be found in the paper. It was also found that the intensity of the action was always proportional to the concentration of the solution, not to the total amount present. It is obvious that some slight loss must take place in such a powerful action, and, by repeated perfusion of a solution through six hearts, it was found that each heart had used 0·002 mg. This amount could not possibly be detected, and hence the reason why it seemed impossible to wash any out, since there was none in sufficient quantity to be detected if it were washed out. It is to be remembered that this quantity taken up by the heart would have no perceptible action if applied to another heart. Straub is inclined to think that the results show that an adsorption at the cell membrane is responsible for the activity, since these glucosides are allied to the saponines, which have great power of lowering surface energy, as we have seen. It will be remembered that, in adsorption, the amount taken up is in proportion to the concentration, just as the action of strophanthin was found to be; and that a certain minimum concentration would be necessary in order that the quantity necessary for action should be adsorbed.

On the view that the cell behaves as a "giant molecule" in the chemical sense, it might be held that one molecule of an active drug would be sufficient to enter into chemical reaction with a cell. It is possible to calculate from Clark's data (1912), together with some which he has kindly given me, what must be the molecular weight of the compound with which strophanthin combines. This glucoside is favourable for the purpose, since it acts directly on the muscle cell. Clark had a more powerful preparation than Straub's, and found that 0·00008 mg. was sufficient to act on 5 mg. of heart muscle (dry weight). The molecular weight of strophanthin is 922, so that a simple proportion gives us the result:—

$$0{\cdot}00008 : 5 = 922 : x,$$

whence we obtain 57,625,000 for the "molecular weight" of the muscle cell, truly a "giant" amongst molecules. The participation in a chemical reaction of such a molecule is difficult to realise, as also how a comparatively small molecule attached at one point should influence the whole of the giant. Naturally, the molecule with which combination takes place may be only one of those present in the cell, but then we have to give up the theory of giant molecules.

The only remaining point of general application to which I would call attention is the nature of "specificity" in relation to these and similar systems. In connection with enzymes, I have discussed the question in "The Nature of Enzyme Action," and shown that the preferential action of an enzyme on a particular substrate is, apparently, only a matter of rate, and if so, that the "lock and key" illustration is not quite appropriate. If a key does not fit it will never open a lock, however long a time be given for it to do so. In fact, it seems to me that such a point of view, met with also in the form of a "moulding to templates," is not applicable to reactions in the organism, if to any chemical reaction at all. The kinetic view of velocities of reaction is more in accordance with facts.

In a previous page (page 60), in speaking of "specific" adsorption, it was pointed out that, in this process, there are innumerable possibilities of interaction of the various forces acting at surface boundaries, without the necessity of calling in the provisions of chemical groups which are to be supposed capable of combination with groups on the substance adsorbed, and with these particular groups only. Attention may also be directed to the work of Barger and W. W. Starling (1915) on the adsorption of iodine by certain organic compounds. This shows itself to be closely related to the chemical composition of these substances and therefore to that of their surfaces. But this relationship does not result in chemical combination nor in abolition of the nature of the process as an adsorption. It would appear that those properties of the surface, such as electric charge and so on, which control the degree of adsorption, are dependent on the chemical nature of the surface. This dependence need cause us no surprise, since the physical properties of a substance, inclusive of surface energy, are so closely related to its chemical composition.

When we have to deal with colloidal solutions, the considerations brought forward by Wolfgang Ostwald (1912) are to the point. We saw (page 107) that, when two colloids have charges of opposite electrical sign, it is usual to find that they mutually precipitate one another. Also that a colloidal solution is precipitated by ions of the opposite electrical sign to themselves. Now, Michaelis and Davidsohn (1912) found that the precipitation phenomena in cases of some "precipitins" and "agglutinins" are nearly independent of the hydrogen ion concentration in the solution, and, therefore, of the electrical charge of the particles. The conclusion drawn is that there is a specific chemical affinity between the substances concerned. But, as Wolfgang Ostwald points out, all that the experiments show is that processes of purely electrical neutralisation are insufficient for the purpose. But it is not to be supposed that electrical charges are the only properties of surfaces that concern colloidal systems. We have only to remember the "coagulation" by neutral salts, by non-electrolytes, by the results of "mechanical" adsorption on surfaces, and so on. Moreover, even when electrical charges are present, the precipitation is not always determined by them. For example, tannin precipitates gelatine better in the presence of acid; gold sols are not necessarily precipitated when deprived of charge. The chief variable, surface tension, is altered, not only by electrical charge, but also by chemical composition, temperature, degree of dispersion, degree of solvation, and so on. The amount of electrolyte required to precipitate sulphur sols, as mentioned above (page 94), depends on the size of the particles.

It is not permissible, in fact, to refer all hitherto unexplained phenomena to chemical relations, as is customary. Substances are frequently assumed to be chemically different because they have some different physical property, although no *chemical* difference can be detected. Tannin, for example, has a higher optical activity in a higher dispersed condition than in a coarsely

dispersed one, and has, therefore, been stated to be a mixture of "chemically different tannins," although no chemical difference has been shown to exist. Wolfgang Ostwald justly insists that the "chemical" conception is here a purely negative one, and that to be satisfied with the fact that the chemical composition of such substances as "immune bodies" is so complex that one may quietly ascribe all their properties to it, is the antithesis of a view leading to progress.

The growth of excised tissues in plasma is of interest in this connection. It was thought at one time that such tissues would only grow in plasma of the same individual, an extreme case of specific relationship. But Walton finds (1914) that this is not the determining factor; any plasma, of the same or another individual, may contain certain substances which inhibit the growth of tissue, together with others which favour growth. The former are destroyed by freezing the plasma for one to three days, the latter by a longer period of freezing, six to eight days. Further, D. and J. G. Thomson (1914) have found that tissue from certain human tumours can be cultivated successfully in the blood-plasma of the fowl, to which has been added extract of embryo chick. Champy et Coca (1914) find the plasma of the cat is toxic for the tissues of the pigeon, while rat tissue grows excellently in tortoise plasma. The toxicity, in fact, is merely accidental. Reference may also be made to the work of Margaret R. Lewis (1915, p. 155) who obtained excellent growth in Locke's Ringer solution.

We may now proceed to refer, briefly, to certain examples of drugs which have a physiological interest.

It is remarkable how great a variety of these active substances are formed by plants. It seems evident that they must be more or less accidental products of chemical change. A very small number would suffice for protection of the plant from being consumed by animals for food. Similar conclusions may be drawn from the occurrence of adrenaline and a substance related to digitalin in the "parotoid" glands of a tropical toad, described by Abel (1911). It is impossible to see the use to a toad of a rise of blood pressure in the animal which attacks it.

Acetyl-choline.—Dale (1914) finds that this substance produces vascular dilatation in extraordinarily small doses, much smaller than those of adrenaline required to raise the blood pressure. The perfused heart of the frog also shows a distinct inhibition with a dilution of one in a hundred millions. It excites especially the nerve endings of the cranial and sacral autonomic systems, causing stimulation of the vagus, secretion of saliva, contraction of the œsophagus, stomach, and intestine, and of the bladder. The effects, although so powerful, last but a short time. The ester is probably hydrolysed into its relatively inert components. It has scarcely any action on the plain muscle known to be innervated by the sympathetic system. Since it produces vascular dilatation, the non-sympathetic origin of vaso-dilators in general seems to be indicated (see also Gaskell, 1916, pp. 62 and 131). Reid Hunt (1918) finds that this drug causes obvious vaso-dilatation in a dose of $2{\cdot}4 \times 10^{-9}$ mg. per kilo. of animal. Its effect is prevented by atropine, contrary to that of the vaso-dilator nerves.

Strychnine.—It is unnecessary here to say more about this alkaloid than to call attention to its peculiar physiological property of converting inhibition into excitation in the phenomena of reciprocal innervation (page 427).

Nicotine.—It was shown by Langley and Dickinson (1889) that nicotine has the property, in moderate doses, of paralysing the nerve cells of sympathetic ganglia, without affecting the peripheral endings of the fibres. The effect appears to be exerted on the synapse, and is not confined to sympathetic ganglia. It serves, therefore, as a valuable means of discovering whether there is any cell station for given fibres in any situation to which the drug can be applied. Langley has made considerable use of it for this purpose. The synapses of different nerves require different doses, and the sensibility of different species of animal differs.

Preceding the paralysis, there is a stage of stimulation, so that, amongst other phenomena, a large rise of arterial pressure results from intravenous injection of nicotine.

Atropine and Pilocarpine.—The interest of these two alkaloids, the first in paralysing secretory nerves, the second in stimulating them, has been referred to

above (page 344). Another useful property of atropine is to paralyse the vagus endings in the heart, and also that of paralysing the mechanism of accommodation in the eye, and causing dilatation of the pupil. Certain peculiarities in the mode of action of pilocarpine have been mentioned above (page 143).

Hyoscine.—This alkaloid, allied to atropine, is of interest in connection with the different activities of the natural alkaloids and their optical isomers. The latter, although not inactive, are less so than the former. Now hyoscine contains two optically active groups, both of which are physiologically active, so that four different isomers exist, each with a different degree of activity. Cushny finds that by assigning to each of the four constituents a definite value, that of each of the four hyoscines can be deduced. The values differ less than those of the two adrenalines. The question has some theoretical interest in connection with the doctrine of fitting to templates or "lock and key." If this were the correct point of view, it would be expected that only one of the isomers would be active, whereas they only differ in degree.

Veratrine.—The peculiar effect of this alkaloid in producing tonic contraction of skeletal muscle has been mentioned above (page 417). Lamm (1911) has described some interesting experiments which throw light on its mode of action. The conclusions which he draws are as follows. When a muscle is immersed in a solution of a salt of veratrine, it takes up small amounts of the poison, probably as free alkaloid, since the effect is more powerful in alkaline solution. A solution can be exhausted by means of a series of muscles. The drug has no effect until the muscle is stimulated independently. If the toxic action is small, the veratrine "tetanus" does not come on until the initial twitch is over. There is no reason to suppose that the kind of fibrillation taking place is due to stimulation of any different kind of substance (sarcoplasm) than that responsible for the twitch. It appears to be due to some kind of reaction between the poison and some product of metabolism of the active muscle. Thus a solution which has served for action on one muscle is found to be increased in activity. If a muscle is disintegrated in a veratrine solution, it is found that apparently *more* alkaloid can be extracted from the mass than was originally present in the solution. It is suggested that the exciting action depends on an increase in permeability of the cell membrane, since calcium salts markedly increase the amount necessary for an effect.

Ergotoxine.—Dale (1906) and Barger and Dale (1907) have obtained from ergot a preparation which has interesting properties. It paralyses those sympathetic endings which have an excitatory function, leaving intact those with an inhibitory function. Both kinds of fibres in the cranial and sacral autonomic nerves are left untouched. Thus the vaso-constrictors of the sympathetic are paralysed, but the inhibitory effect of the splanchnics on the small intestine is not affected. Adrenaline, after ergotoxine, causes a fall of blood pressure, perhaps due to the unantagonised effect on the capillaries described by Dale and Richards (see page 706 above).

Cushny's book (1910, 2) will be found to contain any further information required by the reader regarding the action of drugs.

Toxins and Antitoxins

Toxins are the poisonous substances produced by micro-organisms. They may pass out into the culture fluid or be retained in the bodies of the microbes, only to be obtained from these by disintegration. Their chemical nature is unknown, since they can only be obtained in such small quantities. They are the substances responsible for the numerous diseases produced by the agency of micro-organisms. Pasteur showed, for example, that a culture of the bacterium of chicken cholera produced the symptoms of the disease, even after filtering off the organisms themselves. For further details, the reader is referred to the book by Burnet (1911).

Along with certain other substances, all of protein nature, so far as reliable

evidence goes, toxins have the property of producing, when injected into the living organism, certain substances which have the power of neutralising their action. These are known as *antitoxins*, or, in general, as *antibodies*, and those substances capable of exciting their production are called their *antigens*. Statements have been made that substances other than proteins may act as antigens; we have seen (page 316) that there is no satisfactory evidence that enzymes can act thus, although some lipoids and glucosides have been said to do so; the evidence, however, that the preparations were free from protein is not sufficiently clear.

The mechanism by which "specific" antitoxins are produced is not yet understood. Very complex colloidal reactions are certainly involved. Two cases may be mentioned. The blood of the crayfish injected into a mouse makes it immune against scorpion toxin; but the crayfish itself is more sensitive to the toxin than the mouse is, and its blood does not protect another crayfish. Rabbits can be immunised against tetanus by inoculation with tetanus bacilli in repeated small doses. Yet their serum has no effect in neutralising the toxin *in vitro*.

Fig. 258. Louis Pasteur.
(From "Scientific Worthies." By permission of Macmillan & Co. Ltd.)

The reader is recommended to consult the papers by Gengou (1908) for various facts to be taken into consideration. The "side-chain" theory of Ehrlich has now ceased to be of much help, otherwise than in the invention of new names, valuable as it has been in the past. The paper by Dean (1917) on the mechanism of serum reaction may be mentioned.

Anaphylaxis

It was found by Portier and Richet (1902) that a toxin could be prepared from the tentacles of the sea anemone which caused intense vascular congestion in the viscera of dogs, leading to death after some hours. They found also that, if a dose insufficient to cause death was given, so that the animal recovered, a second dose of only one-twentieth the amount of the first, if given subsequently, caused extremely severe symptoms, vomiting, diarrhœa, paralysis, and so on.

This effect was obtained only if a certain minimum number of days (eight to twelve) had elapsed between the first and second injections. It will be remembered that, in the usual process of immunisation to a toxin, injections are given at intervals of about four days, without the appearance of severe symptoms, so that the manifestation of this supersensitive state, called by Richet, "*anaphylaxis*," is prevented by the development of the ordinary state of immunity. We shall see presently that the two processes are intimately connected.

A remarkable fact is that non-toxic substances, such as egg-white, are also able to produce severe symptoms of anaphylaxis. But a necessary condition seems to be that of the colloidal state; a similar condition applies to the property of acting as antigens, in general.

What evidence have we as to the nature of this interesting phenomenon? The monograph by Richet (1912) gives a good general account of the subject, but the most important experimental work is that of Dale (1912). The value of these investigations consists in the fact that they were made chiefly on an isolated organ, the virgin uterus of the guinea-pig, so that the conditions could be

accurately controlled; the results are of great importance, not only in the interpretation of anaphylaxis, but in the theory of immunity in general.

It is evident, to begin with, that some change is produced in the blood, by which the tissue cells are affected. In different animals, the actual symptoms vary according to the particular organ most sensitive to these changes in the blood. In the guinea-pig, the bronchial muscle is most affected, in the dog, the liver and intestine. We have seen that a considerable time is necessary for these changes to be produced in the blood, but, once produced, the blood of a sensitised animal can bring about anaphylactic shock in a normal one, immediately, as was shown by Manwaring (1910). At the same time, the anaphylactic substance, or influence, becomes fixed or adsorbed on the tissue cells, so that, as Dale shows, the excised and washed uterus of an anaphylactic

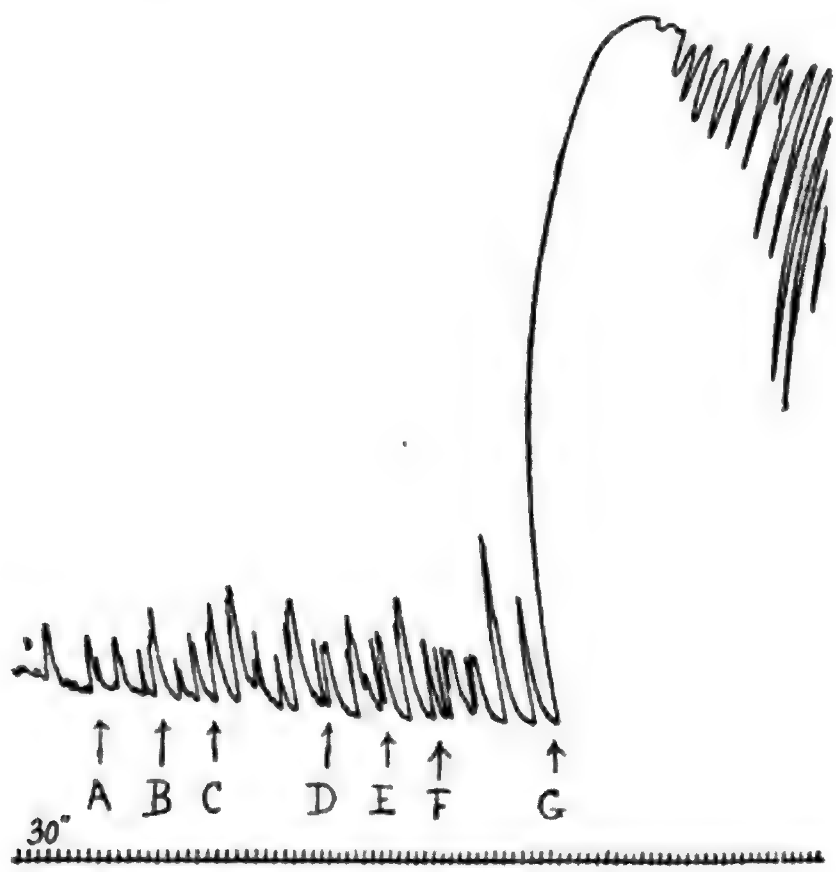


FIG. 259. ANAPHYLAXIS IN EXCISED UTERUS OF GUINEA-PIG.

Sensitised with $\frac{1}{100}$th c.c. horse serum.

A, 0·1 c.c. sheep serum.
B, ,, cat serum.
C, ,, rabbit serum.
D, ,, dog serum.
E, ,, human serum.
F, ,, egg-white.
G, ,, horse serum.

Powerful contraction with the antigen serum, but with no other serum nor with egg-white.

(Dale, 1912, Fig. 26.)

animal, suspended in Ringer's solution, is itself sensitive to the antigen. Fig. 259 shows the effect, together with the striking "specificity" of the reaction. The organ was taken from a guinea-pig, sensitised by an injection of horse serum, fourteen days previously. It gives no response to a small dose of the serum of the sheep, cat, rabbit, dog, or man, nor to egg-white, but a powerful contraction to a similar dose of horse serum, the antigen itself.

Now, there are two points to be cleared out of the way before we proceed. Fresh serum of all animals contains a toxic substance, which causes contraction of smooth muscle; this property diminishes considerably as the serum is kept and has nothing to do with the anaphylactic reaction. A much larger dose has to be given; but, in the testing of the anaphylactic phenomenon, the fact must be remembered. The curves of Fig. 259 show that the doses given have no effect of this kind. Secondly, it might be supposed that the normal guinea pig uterus

is particularly sensitive to horse serum, as compared with other sera. This is found not to be the case; a dose of horse serum, just as large as that of any other serum, is required to cause contraction of the normal uterus.

Although the specificity of the reaction appears to be so great, and is, in fact, under proper conditions, sufficiently so to serve as a delicate test for the antigen, there are some facts which show that it is really only of a quantitative nature, and also that the phenomena of specific *immunisation* share this characteristic. Dale finds (p. 188) that if the guinea-pigs are sensitised simultaneously to horse serum, sheep serum, and egg-white, as can be done, the sensitiveness to horse serum, although present, is comparatively low. To understand the further evidence, we must consider the fact of *desensitisation*. Suppose a uterus, sensitive to horse serum, has been tested with a fairly large dose and has given a powerful contraction, it is found to be incapable of response to a second dose. It is evident that some kind of reaction has taken place between the sensitised tissue and the antigen,

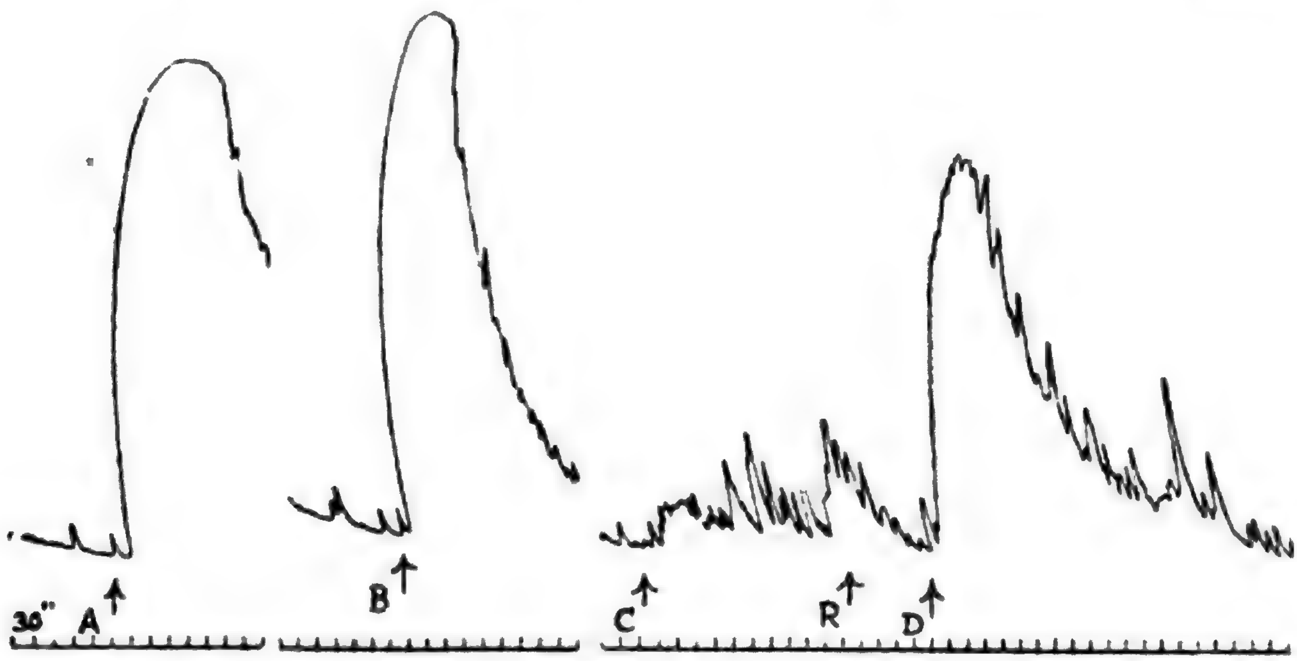


FIG. 260. IMMUNISED UTERUS SENSITIVE TO SERA OTHER THAN THE ANTIGEN. DESENSITISATION.

Guinea-pig immunised to horse serum by ten injections.
Tested for anaphylaxis twenty-four days after the last injection. Perfused.
A, 0·5 c.c. sheep serum causes contraction and subsequent desensitisation for a further dose at *C*.
B, (After change of bath) 0·1 c.c. horse serum.
C, (After further change of bath) 0·5 c.c. sheep serum.
R, Fresh Ringer solution.
D, 0·5 c.c. horse serum (the first dose was not large enough to effect complete desensitisation).

(Dale, 1912, Fig. 12.)

in which the sensitiveness of the tissue has been abolished. It appears that the antigen has fixed itself in such a way as to prevent the action of further doses. Take, next, the above uterus sensitised to three antigens. Treat it first with sheep serum, so as to desensitise it to this antigen. Its sensibility to horse serum is now also very small, but is unaffected towards egg-white. If, however, the uterus be first desensitised to egg-white, it is found to be desensitised to both the others. In another experiment, it was found that, by first treating with sheep serum (p. 189), the sensibility to egg-white was greatly diminished, though not abolished. Further evidence is afforded by the behaviour of the uterus of animals *immunised* by repeated doses of horse serum. It is, of course, well known that such animals are no longer sensitive to a small dose of the antigen. But the isolated uterus itself still remains capable of anaphylactic reaction. A series of ten injections of horse serum was given, at first at three days' interval, that is, during the "incubation period" of anaphylaxis. On testing the uterus, it was found to be sensitive to *sheep* serum, though less so than to horse serum. It was desensitised, however, to sheep serum in the process, so that the phenomenon was a genuine anaphylaxis (see Fig. 260). These facts seem to exclude explanation by specific "chemo-receptors." If there is a particular receptor for egg-white, for

example, which is taken possession of in treatment with the antigen, why is the tissue thereby made insensitive to sheep and horse serum also?

If three months were allowed to elapse after the last injection of horse serum, although the immunity of the whole animal was still present, the anaphylactic reaction of the excised uterus had nearly disappeared. This latter kind of immunity, by disappearance of sensitiveness, must not, of course, be confused with the immunity conferred by the presence of antibodies in the blood, which are supposed to neutralise the antigen before it has attacked the tissues.

It has been already mentioned that the blood of an anaphylactic animal can produce the state in a normal one, so that Dale made experiments to see how far a normal uterus could be sensitised by treatment with serum of a sensitised animal. It was found comparatively easy to resensitise an anaphylactic uterus which had been desensitised. Mere contact for some hours with not too dilute a serum of a sensitised animal was sufficient. Perfusion of a normal uterus with diluted serum from sensitive animals also conferred a decided sensitisation. It is evident, then, that interaction with other organs is not necessary for the reaction in the guinea-pig, and it was found that the bronchial spasm could be produced even when the liver and intestines were removed. In the dog, the reaction in the liver produces some secondary reactions, by which toxic substances are sent into the blood, causing exaggeration of the symptoms.

Certain theories have been suggested in explanation of the phenomenon. That there is some sort of interaction between the antigen and some constituents of the serum is indicated by the results of Anderson and Frost (1910), who found that serum from a sensitised animal, after digestion with antigen, produced symptoms resembling anaphylactic shock when injected into normal animals. Experiments of this kind led to the theory of a kind of proteolytic digestion, but Doerr (1912, p. 337) has shown that similar effects can be obtained from serum that has been digested with kaolin or kieselguhr. Further, Doerr and Moldovan (1912) find that many of the symptoms of anaphylactic shock can be obtained by intravenous injection of some inorganic colloids, such as silica.

According to Dale, these latter experiments indicate the direction in which to look for a more satisfactory explanation than the protein digestion theory. In addition to the facts mentioned, the absence of any perceptible latent period, the sudden onset, and the gradual decline are quite unlike any enzyme action. The effect, in fact, is like that of a powerful stimulating drug, such as β-iminazolylethylamine.

The action of inorganic colloids is usually to give rise to mutual precipitation or aggregation, when mixed with protein solutions. Dale's experiments, as he points out, show that the colloidal interaction must take place on the muscle fibres themselves and need not actually go so far as precipitation. In the light of the work of Lillie and others (page 398), on the increase of permeability in the state of excitation, it seems highly probable that the contraction resulting from the interaction of the sensitised muscle fibre with the antigen is a consequence of increased permeability of the cell membrane. This view is supported by the fact that the presence of calcium salts tends to oppose the reaction, as would be expected from their relation to colloidal processes. As Dale says (p. 221), "The action of the antigen in extreme dilutions, the saturation of the antibody (desensitisation), the cessation of the effect when the union of the antibody and antigen may be supposed to be complete, all find their reasonable explanation."

Dale (1920) points out the resemblance of the antibody causing anaphylaxis to that which precipitates its antigen, the so-called "precipitin." "The remarkable difference, in physiological response to the antigen, between the anaphylactic and the immune animal depends on the different distribution of the antibody between the blood and the cells." The reaction in the former case takes place in the cells, while an excess of antibody in the blood protects the cells by removing the antigen from the sphere of action before it reaches them. The different type of effects in different species of animals is that shown to various protein derivatives, including histamine. Anaphylaxis, then, is not the opposite of immunity, but is "the physiological response of an animal in a certain phase of immunity to the artificial test which we impose." For further particulars and criticism of other hypotheses, the original lecture should be consulted.

I have spent some time in the description of these experiments because they

apply not only to anaphylaxis, but to immunity in general. In connection with them, the fundamental paper by Wooldridge on "Chemical Protection" (1888) should be referred to. The main result of this work was to show that a solution of a "tissue-fibrinogen" could confer specific immunity. The solution was prepared from the thymus of normal rabbits; it was partly coagulated by boiling and pressed through fine linen, so as to obtain a very fine suspension. When injected, this suspension was found to confer immunity against anthrax. Thus, it was shown "that immunity can be obtained, not, as had previously been supposed, only by the inoculation of attenuated micro-organisms or their products, but by the administration or introduction into the system of a chemical substance which had never come into relation with or was in no sense a product of the life of a micro-organism" (Introduction to "Collected Papers," p. 28).

Phagocytosis and "Opsonins"

Certain specific substances have been described which are supposed to increase the taking up of micro-organisms by leucocytes. This is said to be done by an action of the micro-organisms of such a nature as to make them attractive to their devourers. The work of Ledingham (1912), already referred to (page 3), has shown that mere agglutination is sufficient for the purpose. Further evidence against the necessity of assuming specific "opsonins" is given by Savchenko and Aristovsky (1912). They show that the optimum reaction of the medium for phagocytosis is the same as that most favourable for the adsorption of the "alexin" by the object of phagocytosis; and that phagocytosis, as showing itself by the mutual attraction (relative surface tension) of the leucocytes and the object, and by the moistening of the object by the protoplasm of the leucocyte, depends on the adsorption of the alexin by the object of the phagocytosis. The meaning of the "alexin" requires further investigation.

SUMMARY

There are a large number of substances, acting powerfully in minute amount, which are of great importance in physiological processes.

One class of these consists of the hormones, or chemical messengers, which are produced in a particular organ, pass into the blood current, and produce effects in distant organs. They provide, therefore, for a chemical co-ordination of the activities of the organism, working side by side with that through the nervous system.

The most typical instance of this kind is the pancreatic secretin. Methods of preparing active secretin solutions, free from the depressor substance, are given in the text.

There is evidence that a similar substance is produced from the pyloric mucous membrane, and excites the secretion of gastric juice.

The internal secretions, formed by ductless glands, as well as by other tissues, belong to the class of hormones.

The remarkable relationship of the medulla of the suprarenal glands and its secretion, adrenaline, to the sympathetic system is discussed in the text.

Certain drugs, such as nicotine and pilocarpine, produce a part of their effects by stimulating the secretion of adrenaline into the blood.

The respiratory centre is stimulated by the increase of hydrogen ion concentration in the blood, due mainly to carbon dioxide, a parahormone, in Gley's sense. There is no satisfactory evidence of its acting as a specific hormone.

The various hormones arising from the sexual glands are responsible for the development of the secondary sexual characters. These hormones appear to be produced by the interstitial cells, not by the generative cells themselves. The corpora lutea of the ovary are responsible for the first stages of uterine hypertrophy and for the growth of the mammary gland.

There are certain "ductless glands," pituitary, thyroid, and thymus, which have a marked influence on growth in general.

Complete removal of the pancreas results in the production of severe diabetes. The structures responsible for the "antidiabetic" hormone are the islets of Langerhans.

There are interrelations between certain of these internal secretions, but, at present, their nature is still very obscure.

Some evidence exists of the production of hormones in plants.

The mode of action of drugs is discussed in the text. The conclusion arrived at is that the chemical structure alone throws very little light on their action. The theory of "chemo receptors" or special receptive side-chains in the cell is found to be contrary to many facts, and to be generally inappropriate. While the cells take up certain alkaloids, such as atropine, which can be regained from them, they do not take up any detectable amounts of strophanthin, which acts in direct proportion to its concentration outside. It is probably adsorbed at the cell membrane.

In the explanation of "specific" action, phenomena due to surface action, and other physical forces, have to be taken into account. In fact, many of the supposed cases of extreme specificity have been found, on inquiry, to be due to factors other than specific chemical relationship.

The properties of certain individual drugs, of physiological interest, are described in the text. Such are, acetyl-choline, strychnine, nicotine, pilocarpine, atropine, hyoscine, veratrine, and ergotoxine.

Certain substances of colloidal nature, probably all of protein constitution, such as the bacterial toxins and foreign proteins, give rise to the production in the blood of antibodies which are capable of neutralising, in various ways, the action of the antigen injected.

If an interval of not less than eight to ten days elapses after an injection of one of these substances, the animal is found to be supersensitive to the antigen (anaphylaxis). If an antigen usually innocuous, such as egg-white, is injected in this sensitive state, severe symptoms are produced.

Under certain conditions, the state of anaphylaxis is very specific, an isolated organ reacting only to the actual antigen alone. But results obtained by Dale indicate that the property is merely one of degree. Organs sensitised by one antigen, under particular conditions, may react to a different substance.

The theory of specific chemo-receptors does not give a satisfactory account of the phenomena. The explanation appears to be in a colloidal precipitation process at the surface of the sensitive cells, by which their semipermeability is more or less destroyed.

Immunity and anaphylaxis are closely related phenomena.

Results have been obtained by Wooldridge, which show that tissue extracts from normal animals can be obtained, which are capable of conferring immunity in a similar way to the specific antitoxin obtained by bacterial inoculation.

The facts connected with the increase of phagocytosis by the so-called "opsonins" are, in all probability, to be explained by adsorption, causing changes in surface tension. They are only to a limited degree specific.

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The same remarks apply to many Japanese names, which are frequently given in their German spelling.

This practice applies, of course, only to languages which do not use the Latin characters; in those languages which do so, French, German, Italian, etc., the names are spelled in their original form.

In order to shorten the titles of journals, I have made use of a certain number of abbreviations, whose meaning will generally be obvious; but their significance will, in any case, be found in the following table:—

Abh., Abhandlung.
Acad., Academy, Académie.
Accad., Accademia.
Akad., Akademie.
Agr., Agriculture and the similar word in other languages.
Amer., American.
Anat., Anatomy or anatomical, or similar.
Ann., Annalen, Annales, Annals.
Anz., Anzeiger.
Arb., Arbeiten.
Arch., Archives, Archiv.
Beitr., Beiträge.
Ber., Berichte.
Bioch., Biochemical, Biochemisch.
Bot., Botany, Botanical, etc.
Chem., Chemical, chemisch.
Chim., Chimie.
Chem. Ber., Ber. der Deutschen chem. Ges.
Clin., Clinical, Clinica.
Compar., Comparative.
C. R., Comptes rendus de l'académie française.
Dtsch., Deutsch.
Entwickl., Entwicklungsmechanik.
Exper., Experimental.
Fol., Folia.
Ges., Gesellschaft.
Hyg., Hygiene.
Immun., Immunitätsforschung.
Inst., Institute.
Internat., International.
Jahrb., Jahrbuch.
Jl., Journal.
Landw., Landwirtschaft.
Med., Medicine, medical, etc.
Micr., Microscopic.
Mikr., Mikroscopisch.
Mon. H., Monatshefte.
Mon. Schr., Monatschrift.
N. F., Neue Folge.
Pathol., Pathology, etc.
Physikal., Physikalisch.
Physiol., Physiology, physiological, physiologie, physiologisch.
Pharmac., Pharmacology.
Pharmakol., Pharmakologie.
Phil. Trans., Philosophical Transactions of the Royal Society.
Proc., Proceedings.
Protist., Protistenkunde.
Psychol., Psychology, etc.
Quart., Quarterly.
Rec., Recueil.
R. S., Royal Society.
Sci., Science, Scienze.
Sitz. Ber., Sitzungsberichte.
Soc., Society, Société, etc.
Soc. Biol., Sociéte de Biologie.
Trans., Transactions.
Trav., Travaux.
Vergl., Vergleichende.
Verh., Verhandlungen.
Vers., Versuchsstation.
Veter., Veterinary, etc.
Wiss., Wissenschaften, wissenschaftlich.
Woch., Wochenschrift.
Zbl., Zentralblatt.
Zool., Zoology, Zoologie.
Zs., Zeitschrift.
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